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CONTENTS

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CANCER RESEARCH

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The Histogenesis of Benzpyrene-Induced Epidermal Tumors in the Mouse*

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The macroscopic and microscopic changes in mouse skin following the external application of tar or of carcinogenic hydrocarbons have been described and reviewed in some detail (8, 13, 66, 70). But though all accounts agree on the main facts, such as the cyclical changes in the hair coat, the induction of epidermal hyperplasia followed by the appearance of warts and later of malignant tumors, disagreement persists on the following points:

1. The specificity of the initial epidermal hyperplasia:

Wolbach (71, 72), Orr (52), and Cramer and Stowell (18) consider the initial epidermal hyperplasia as nonspecific and as similar to hyperplasia induced by noncarcinogenic irritants; Pullinger (57, 58) stresses the specificity of this reaction; Twort and Twort (68) hint at a possible distinction between the hyperplasia caused by oleic acid and that produced by noncarcinogenic skin irritants; and Paletta, Cowdry, and Lischer (56) report some differences between simple regenerative and precancerous hyperplasia.

2. The primary effects of the carcinogens:

Wolbach (71, 72), Orr (52), and Cramer and Stowell (18) consider the epidermal hyperplasia as regenerative and as subsequent to primary deleterious effects of the carcinogens; Pullinger (57, 58) finds little evidence of initial degenerative changes with fairly large doses of carcinogenic hydrocarbons applied in nonirritant solvents; Kuklianskis (36) reports the absence of cellular degeneration in mouse organs up to 38 hours after the injection of various carcinogens; and Cooper and Reller (15, 64) report a significant rise in mitotic activity within 48 hours of application of carcinogens without finding any prior tissue damage. On the other hand, undoubted growth-inhibiting effects of a specific nature were obtained by Haddow and others (31, 39) after the injection of carcinogens. Tests made on unicellular organisms (51, 67), tissue cultures (23, 24, 44), frog embryos (10), and other biological material (28, 29, 32, 53, 63), seem to indicate that primarily stimulating as well as deleterious effects occur depending on dose, concentration, and solvent used, and on the test object chosen.

3. The permanency of the initial changes and their dependence on a specific sensitivity of the epidermis:

While some mice react to a single application of carcinogenic hydrocarbons with the formation of warts and of malignant tumors (19, 37, 46), others require repeated paintings preferably at optimal intervals (17, 20) to produce the same result. In these animals warts and epidermal hyperplasia may regress in the absence of further applications. Nonspecific stimuli, varying from mechanical trauma (21, 22, 43, 45, 59, 65) to certain chemical irritants (3, 4), thermal agents (2, 42), or ionizing radiations (50) may maintain epidermal hyperplasia and warts initiated by the carcinogen, and even induce malignancy. Epidermal sensitivity to tar or carcinogenic hydrocarbons varies with species, strain, individual, and the region in the individual (7, 13, 27-29, 66, 68, 70). Since no parallel degree of variation in sensitivity is reported for subcutaneous tissue, the biological basis for the sensitivity variation should be correlated with the epidermal structure. Yamagiwa and his school (70) describe a difference in skin reaction to tar in rabbits and mice, and attribute the response in mice to the epidermis itself and in rabbits mainly to the hair follicles. Jonkhoff (35), on the other hand, demonstrates a definite correlation between periods of hair growth and periods of growth of papillomas in mice.

In the hope of obtaining some definite information on these points and on their significance in experimental carcinogenesis, a quantitative histological investigation of the effects of carcinogenic hydrocarbons and of relatively unspecific skin irritants in animals of a sensitive (mice) and of a resistant (rats) species was undertaken.

*Because of the difficulties of international communication the author has not read proof of this article.

MATERIAL AND METHODS

The mice used for these experiments were derived from an albino stock originally obtained from the National Institute, in Hampstead, and carried on in our laboratory by brother-sister matings. During a period of 5 years no spontaneous skin tumors have been observed in this strain. For the painting experiments litter mates of about 2 months, separated as to sex, were used. The rats were of mixed stock, and about 2 months old at the beginning of the experiment.

Experimental Procedure

The solutions were applied to the interscapular region of the animals by means of a doubly bent pipette. In the mice the hairs were not clipped or shaved before or during the experiments, while in the rats the solutions were applied to the shaved skin. About 5 to 6 drops of the solutions were applied at every painting. The following experiments were made:

(a) On mice.—1. A single painting of a 1 per cent solution of benzpyrene (La Roche) in acetone was applied to 36 mice. The painted skin area was excised at intervals in 2 mice at a time and fixed for histological examination.

2. A 1 per cent solution of benzpyrene in acetone (as above) was applied at weekly intervals to a great number of mice and the treated area excised. The findings in 86 mice thus treated will be considered in this paper.

3. A group of 10 mice was painted once weekly with a 50 per cent solution of turpentine in acetone for 5 months, and individual animals of this group were killed at intervals of 7 days to 9 months.

4. A group of 10 mice was treated with a solution of pure acetone once weekly for 5 months, and all the animals were killed at the end of that period.

5. A group of 10 mice was depilated with a barium sulphide cream (Veet), and individual animals were killed at daily intervals.

(b) On rats.—1. A group of 12 rats was shaved and treated once weekly with a 1 per cent solution of benzpyrene in acetone. Individual animals were killed during the next 3 weeks and the treated area was excised.

2. A group of 7 rats were shaved and individual animals were killed as controls for Experiment 1.

3. A group of 12 rats was painted once weekly with a 1 per cent solution of benzpyrene in acetone for 15 months and then killed.

In most of, though not all, the animals an additional piece of skin from the flank was fixed as control. It was found necessary to study in some detail the postnatal development of the skin in mice and rats, and for this purpose skin from various areas of 48 mice and 17 rats was fixed at daily, weekly, and monthly intervals. Fixation was always carried out 1 or 2 hours before noon.

Histological Technic

The excised skin, stretched on pieces of filter paper, was fixed in Zenker's solution or in Susa-mixture. While the pieces were being carried through the alcohols for paraffin embedding each was halved in a longitudinal direction. One half was subsequently sectioned longitudinally right through the painted area, while the other half was cut at right angles. Serial sections cut at 10μ were stained with hematoxylin-eosin, Heidenhain's Azan, carmalum-orange G-aniline blue, Weigert's elastica stain counter-stained with carmalum, by Feulgen's method counter-stained with a mixture of light green and naphthol green, or by Wilder's method.

RESULTS

I. Histological Analysis of Normal Mouse Skin and Its Postnatal Development

Before the experimental results are described it is necessary to consider the histological characteristics of normal mouse skin. In the adult mouse the epidermis of the interscapular region is very thin, and composed of 1 or 2 layers of cells covered by some layers of keratin. This thin layer of viable cells is described as undifferentiated (18, 57, 58) and formed by 1 cell type only (16); the appearance of many cell layers following the application of carcinogens is consequently regarded as a process of differentiation (18, 57). A consideration of the postnatal development of normal mouse skin, however, leads to a different interpretation of the normal histological structure of the adult skin.

At birth and during the first postnatal days the mouse epidermis shows distinct layer formation with a fully developed stratum basale, spinosum, granulosum, and corneum (Figs. 1a and b). At this time the hair follicles are in the process of producing the first hair coat (30). With the growth of the first pelage the number of epidermal layers is reduced by the shedding of the keratinized layers and the contraction into a single layer of the stratum basale, spinosum, and granulosum. This latter process is gradual and not quite uniform. Thus even in the adult epidermis some areas show 2 or more layers, of which the basal is composed of basal cells proper and of spinous cells, while the more superficial layer is formed by stratum granulosum. The basal cells proper are recognizable by their small amount of foamy cytoplasm and by their indistinct cell walls, while the cytoplasm of spinous cells is more condensed, more eosinophilic, and delimited by a distinct cell wall (Figs. 2a and b). These cellular characteristics are

equally obvious in the many-layered regenerating epidermis or in a depilated epidermis.

The presence of keratinized layers in the normal adult mouse skin, as well as the more obvious changes brought about by experimental conditions, indicates that in the mouse, as in most mammals, the process of keratinization proceeds by way of a stratum spinosum and granulosum. But instead of forming separate layers, the spinous cells and basal cells proper are contained in a single layer except in regions with reduced numbers of hairs (pads, ears, some areas in the abdominal skin, etc.) where the epidermis is thicker and its layering more obvious. The formation of a smooth hair coat in the interscapular region, which acts in a protective manner, is correlated with the reduction in number of epidermal cells and of epidermal layering.

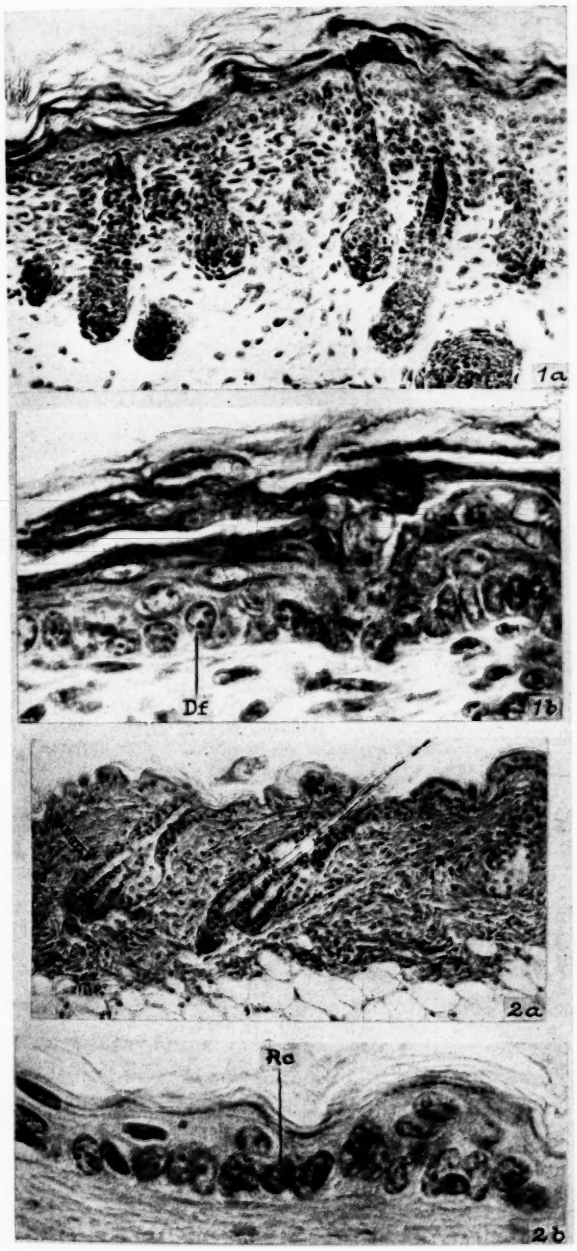


FIG. 1.—Normal mouse skin, interscapular area, 24 hours after birth. (a) Note stratification of epidermis, dense spacing of hair follicles, early formation of hairs and fibrous tissues in dermis. Mag. × 165. (b) Stratum corneum, granulosum, spinosum, and basale can be distinguished. Basal layer contains differentiating cells (Df) of same type as that seen in stratum spinosum. Mag. × 648.

FIG. 2.—Normal skin, 66 days after birth. (a) Hair coat and dermis fully developed. Epidermal layering reduced. Mag. × 58.5. (b) All epidermal layers can be identified on right. In center of basal layer a group of 3 resting cells (Rc) is recognizable by deeply staining nucleus, indistinct outline of cell, and small amount of cytoplasm. Mag. × 825.

The duration of the keratinization process in the adult mouse, *i.e.*, the average life span of an epidermal cell from its mitosis to its casting off as keratinized debris, can be calculated roughly from the following data: (a) the mitotic index for the interscapular region is 0.2 per cent in our material; Champy and Vasiliu (11) find 0.2-0.3 per cent, while Cooper and Franklin (14) calculate 0.11-0.14 per cent as an average over a day for the ear of the mouse. (b) The average duration of mitosis is given as approximately 1 hour by numerous authors (26, 38, 40, 41). Assuming that mitotic cells replace only keratinizing cells, and that the total number of cells for a given area of the adult epidermis remains constant, the life span of the average epidermal cell, *i.e.*, the duration of keratinization, can be calculated as approximately 21 days. This period is of the same order as that of the hair cycle in the mouse (3 to 4 weeks).

For the *quantitative histological analysis* of the normal epidermis, as well as of epidermis subjected to experimental conditions, the most active areas are selected. These regions, characterized by the thickness of the basal layers and the number of mitotic cells, include the regenerating edges of healing ulcers. Such active areas are most likely to contribute directly to the further reaction of the skin, and in particular to the formation of warts and tumors. Necrotic and ulcerated areas have reached the limit of their developmental potentialities and can contribute only indirectly, if at all, to any further reaction.

Since the most active skin region is surrounded by a zone of gradually diminishing reaction that merges at the periphery into the normal untreated skin, the reaction of any treated and surviving skin area must range quantitatively between that of the most active area and that of the normal untreated skin.

Cell counts of selected areas were made in straight fields of epidermis measuring 0.45 mm. in length in longitudinal sections cut at 10μ. Hair follicles and their mouths were excluded from the counts. With the exception of keratinized debris and of stratum granulosum cells with faded nuclei, all cells of the selected area were counted in 1 of the 4 following groups:

(a) Mitotic cells (M) of all stages from prophase to telophase.

(b) Degenerating cells (Dg), *i.e.*, cells in the process of nuclear pyknosis, karyorrhexis, and karyolysis.

(c) Resting cells (RC), *i.e.*, basal cells proper that are capable of division and have not yet embarked on keratinization. They are recognized by their large and deeply staining nuclei; by their sparse, foamy, and basophilic cytoplasm; and by their ill-defined cell boundaries.

(d) Differentiating cells (Df), *i.e.*, cells of the stratum spinosum and granulosum respectively. These have distinct cell walls, greater amounts of more condensed and eosinophilic cytoplasm, lighter staining nuclei, and, in the case of stratum granulosum cells, keratohyaline granules.

1.2 mm). At the end of the first month the number of follicles for the same unit is reduced to 5 for longitudinal and to 10 for transverse sections. The hairs are thus not as densely spaced in the direction in which they overlap. This differential spacing of the hair follicles coincides with the distribution of the epidermal cells into fewer layers.

II. Control Observations on Mouse Skin Treated with (a) Acetone; (b) a Chemical Depilatory; (c) a Nonspecific Irritant

(*a*) *Acetone painting.*—Applied once weekly for a period of 112 days acetone failed to produce any notable macroscopic or microscopic changes in the painted area. The cell counts do not show any significant deviation from those of untreated animals of the same age.

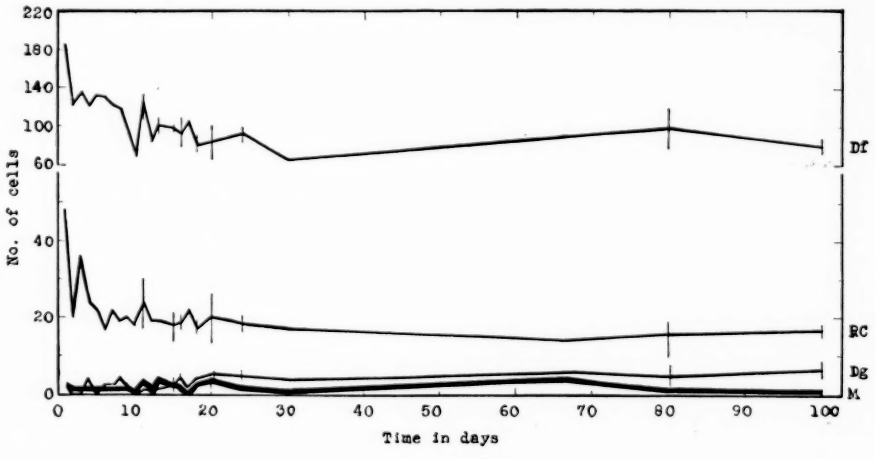


Chart I

The results of the quantitative analysis of the normal postnatal development of the interscapular epidermis are given in Chart I. The average cell count for the unit length of skin is plotted against time. The extent of variation between different animals of the same age is indicated by the vertical lines (absolute maximal and minimal count). Throughout life mitotic and degenerative activity show some fluctuation (other than the diurnal rhythm), caused presumably by minor traumas or parasites. The fluctuations in number of resting and differentiating cells disappear virtually by the end of the first month, and a fairly constant level is maintained subsequently. The initial decrease in number of resting and differentiating cells is correlated with the development of the first hair coat (see above) and with the reduction of epidermal layering (30). During the first period of hair growth a differential spacing of hair follicles is noticed. At birth the number of follicles per unit length of skin is equal in longitudinal and transverse sections (on the average 18 per unit length of

(*b*) *Chemical depilation.*—A barium sulphide cream, applied for 6 minutes to the bathed skin and removed with a wooden spatula, causes a complete and smooth epilation by breaking the hairs at the epidermal level. Newly grown hairs are seen on the sixth day and the whole treated area is covered by short hairs on the tenth day.

Chemical depilation is followed within 2 hours by a slight epidermal thickening and by more definite thickening within 24 hours (Chart II). This increase in cell numbers is referable mainly to spinous cells (Df), which subsequently become stratum granulosum cells (third day) and stratum corneum cells (fifth day). As such they are excluded from the count, although a thickened layer of keratin remains noticeable for at least 10 days. The degenerate and resting cell counts are slightly, though not significantly, raised for the first 3 days, while the mitotic count remains

well within the limits of normal variation. The dermis is slightly infiltrated on the first day.

The early epidermal thickening, unaccompanied by increased epidermal mitotic activity, must be attributed to cellular migration from the hair follicles (18, 55). The hair bulbs show distinct mitotic activity during the first 3 days. But since mitotic activity in the hair bulbs varies with the phase in the hair cycle, and since it provides cells both for hair formation and for migration into the epidermis, it is impossible to correlate quantitatively the number of migrating cells with the increase in mitotic activity of the hair follicles.

(*c*) *Repeated weekly paintings with a 50 per cent solution of turpentine in acetone.*—These caused a slowly progressing epilation in the fourth week, followed by extensive ulceration in the sixth and seventh weeks. The ulcerations healed subsequently, and the hair coat was restored.

In histological specimens taken at weekly intervals the following changes are noticed: During the first 3 to 4 weeks a slight degree of epidermal thickening is associated with a fairly well marked inflammatory reaction in the dermis and with rarefaction of subepidermal collagenous fibers. By the 28th day the epidermis is pitted with numerous small ulcers, the dermis is highly infiltrated by round cells, the subepidermal collagenous fibers are rarefied, and the absence of elastic fibers indicates the formation of minute scars. Some of the hair follicles are epilated, though retaining their usual shape, and the number of mitotic cells indicates their regenerative activity (Fig. 3a). The epidermis at the edge of the ulcers is thickened (Fig. 3b). After 7 weeks most of the ulcers are healed and a new hair coat is formed. Epidermal thickening from adherent scurf persists for some time. The round cell infiltration of the dermis is replaced by fibroblasts, which form a new layer of subepidermal collagenous fibers that is complete by the tenth week.

The quantitative analysis of the epidermal reaction is given in Chart III. During the first 3 weeks a rise in the resting (RC) and degenerate (Dg) cell count is not correlated with an increased epidermal mitotic (M) activity or with an increase in the number of differentiating (Df) cells. Cellular migration from the hair follicles must be responsible for the replacement of degenerate cells and the epidermal thickening of this initial period. In the subsequent period of raised mitotic activity in the epidermis more resting cells are converted into differentiating cells, though the absolute count of resting cells remains constant. During this phase, which coincides with the onset of epilatory changes, the supply of epidermal cells is provided not only by the hair follicles but also by the epidermis itself. The differentiating cells disappear from the count after 7 weeks by becoming keratinized. During the period of ulceration even the most actively regenerating skin areas show many degenerating cells, which disappear with the healing of the ulcers. After 10 weeks the cell counts return to normal proportions.

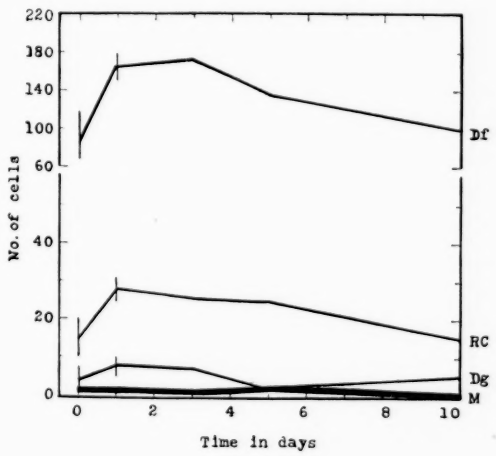


CHART II

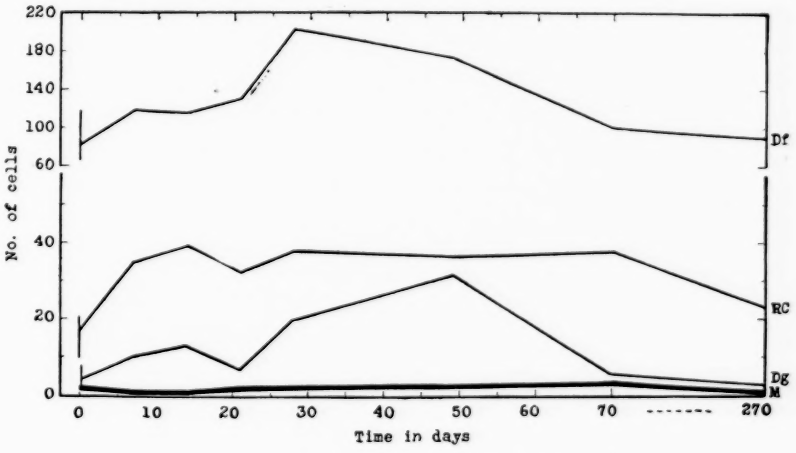
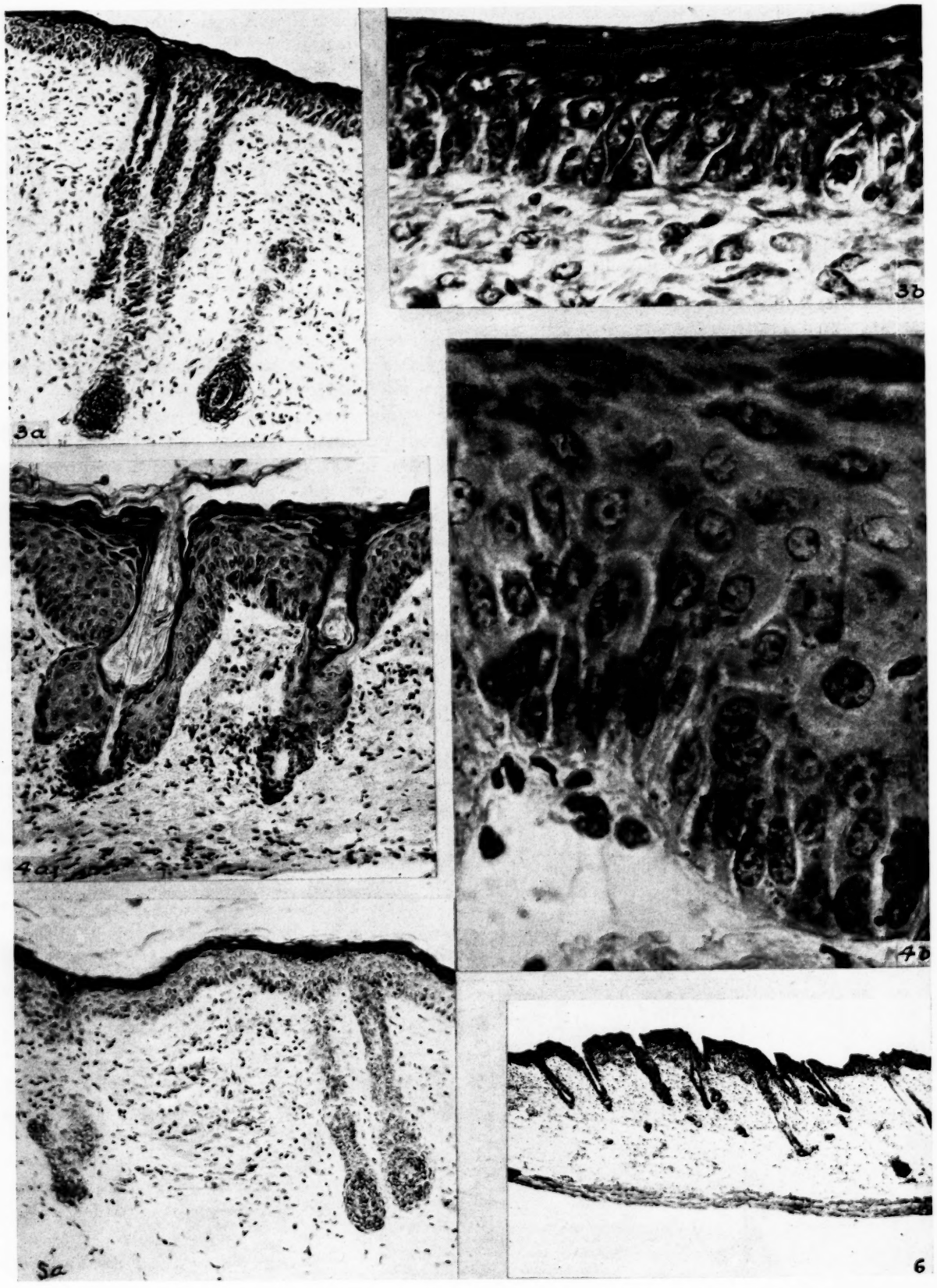


CHART III



FIGS. 3A-6

The epidermal thickening is thus caused in the initial period by cellular migration and subsequently also by increased epidermal mitotic activity.

III. Observations on Benzpyrene-Treated Animals

(*a*) *The reaction of mouse skin to a single application.*—After the rapid evaporation of the acetone the benzpyrene powder remains visible as a yellow stain on the hairs for about 24 hours. In some mice epilatory changes may begin on the third day and a new hair coat be formed by the end of the third week, while in others epilation may start in the second or third week and a new hair coat be formed in the fifth to seventh week. No ulceration, wart, or tumor formation has been observed in this series.

In histological specimens taken 0.5, 2, 4, 6, 8, and 10 hours after application no obvious changes are noticed. Some of the animals examined after 15 and 18 hours show vascular dilatation and a slight degree of round cell infiltration in the deeper dermal layers, and minute preulcerative areas in the epidermis. In these foci the basal cell layer has disappeared and the dermis is covered by keratin only, but there is not yet any round cell infiltration and the adjacent epidermis is thickened. These minute preulcerative lesions seem to heal very quickly, since there is no trace of them in specimens taken after 24 hours or later.

The epidermis thickens considerably on the second day, and this thickening persists in varying degree for 8 to 9 weeks. It is caused at first by an increase in the number of cells in the stratum basale and spinosum, followed on the fourth day by a thickening of the stratum granulosum, which is converted into stratum corneum on the seventh day. Particularly during the first week some of the cells in the stratum spinosum are greatly enlarged.

The epidermal changes are closely related to changes in the hair follicles and sebaceous glands: thickening and keratinization occur in the mouths of the follicles on the second day, extend down to the orifices of the sebaceous glands on the third, and involve the hair bulbs on the fourth day. The thickening of the epithelial hair sheaths results from multiplication of the cells by mitosis, from an increase in cell size, and also from a shortening of the follicles. The increased keratinization of the epithelial sheaths, combined with the shortening of the follicles, forces out the hair (Figs. 4a and b). This process may be complete as early as the third or fourth day, but there is great regional variation as regards epilation, so that some hair follicles may be closed by keratin plugs while some are not yet affected and others have started to regenerate. Usually the shortened and epilated hair follicles remain closed by keratin plugs up to the ninth or 11th day, when they become elongated and show active signs of regeneration leading to the production of new hairs by about the 14th day (Fig. 5). Sebaceous gland cells undergo a squamous metaplasia from the third day onwards when they resemble spinous cells, which subsequently keratinize. This change is probably consequent on the epilatory change rather than a direct effect of the carcinogen. With the regeneration of hairs new sebaceous glands are formed by basal cells at the orifices of the glands, usually by the 14th day.

In the same area the conversion of stratum spinosum into stratum granulosum and of the latter into stratum corneum as a rule occurs simultaneously in the epidermis, hair follicles, and the metaplastic sebaceous glands. As with the epidermal changes, occasional areas of epilatory or regenerative activity in the hair follicles may be visible for about 8 or 9 weeks. Dermal changes, consisting of vascular dilatation, round cell infiltration, and slight rarefaction of subepidermal collagenous fibers are seen for roughly the same period.

The results of a quantitative analysis of the epidermal reaction are given in Charts IV and V. During

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DESCRIPTION OF FIGURES 3A TO 6

Fig. 3.—Mouse skin painted 4 times at weekly intervals with a mixture of turpentine and acetone, and fixed on 28th day of experiment. (a) Hairs absent in treated areas, but the elongated hair follicles are beginning to form new hairs. Dermis infiltrated by round cells. This area is adjacent to a small ulcer. Mag. × 135. (b) Thickened epidermis shows distinct layering. Basal layer contains differentiating cells as well as resting cells and a mitotic cell. Mag. × 825.

Fig. 4.—Mouse skin, 5 days after single application of benzpyrene in acetone solution. (a) The epidermal thickening is due to an increase in number and size of cells. Hair follicles shortened and their mouths closed by keratin plugs. Sebaceous glands undergoing squamous metaplasia except for the basal layer on their outside. Inflammatory dermal reaction extends to epidermis. Mag. × 135. (b) Note increase in cell size and clear distinction between differentiating cells (stratum granulosum and spinosum) and resting cells, of which the basal layer is almost entirely composed. Mag. × 825.

Fig. 5.—Mouse skin painted twice with benzpyrene and fixed on 13th day of experiment. This area still epilated but elongated hair follicles are beginning to form new hairs. Epidermal hyperplasia not so distinct, because of conversion of stratum granulosum into stratum corneum. Inflammation of dermis only slight. Mag. × 135.

Fig. 6.—Mouse skin painted with benzpyrene at weekly intervals and fixed on 100th day of experiment. Epidermis highly hyperplastic. Areas like these form the center of warts. Mag. × 38.

the first 24 hours (Chart IV) the number of differentiating (Df) and resting (RC) cells is not significantly altered. An almost immediate rise in the number of degenerate (Dg) cells is followed within 6 hours by a progressive increase in mitotic activity (M). Calculations based on a duration of mitosis of 1 hour and of degeneration of 7 hours (38) show that mitotic activity in the epidermis accounts satisfactorily for the increased number of resting cells on the second day, and for the subsequent increase

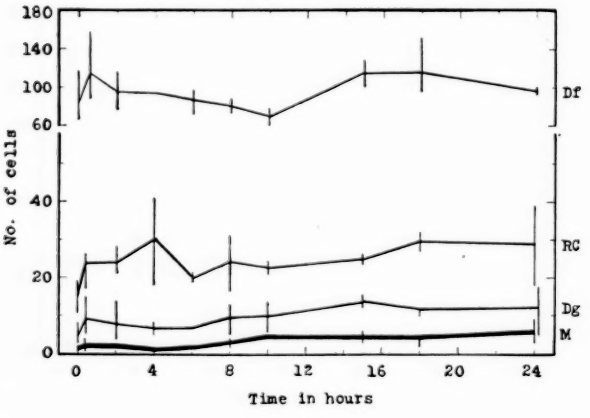

CHART IV

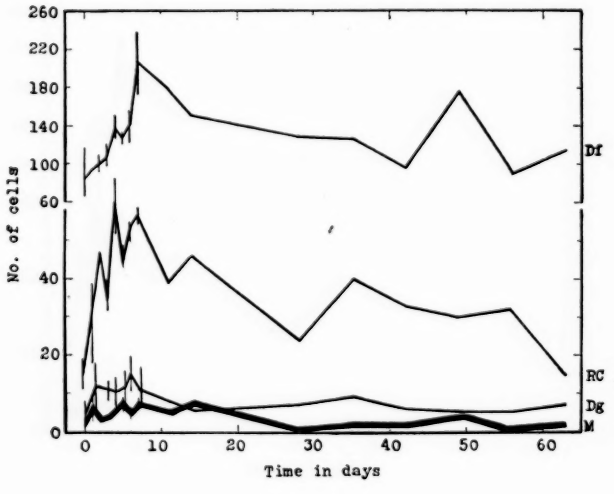

CHART V

in the number of differentiating cells (from the third day onwards). A peak in the resting cell count is reached on the fourth day and is followed by a peak value for the differentiating cell count on the seventh day (Chart V). At this time the stratum granulosum is converted into stratum corneum, which for some time may remain adherent to the skin as scurf but is excluded from the cell counts. Resting and differentiating cell counts remain at a fairly high level for another 5 weeks, when they return slowly to normal proportions. The mitotic activity reaches its normal value after about 3 weeks, while the count of degenerate cells regains the upper limits of normal variation at about the seventh day, but remains at the relatively high level throughout the further period of observation. The return to normal proportions of the cell counts is subject to individual variation, and while in some mice it occurs as early as 6 weeks after painting, others may show altered counts after 9 weeks.

To summarize, the epidermal thickening that ensues upon benzpyrene painting results from an early rise in mitotic activity, followed subsequently by a distinct rise first in the resting and later in the differentiating cell count. There is no evidence that cellular migration from the hair follicles contributes to the increase in epidermal thickness. There is also an almost immediate, though not very definite, increase in the number of degenerate cells. Mitotic activity is not inhibited at any stage following painting.

(*b*) *The reaction of mouse skin to repeated applications at weekly intervals.*—The macroscopic changes consist of a cycle of epilation and regeneration of the hair coat followed by, or later coinciding with, the formation of warts and of tumors. Jonkhoff's (35) data on tarred mice suggest a hair cycle in the treated animals of about 22 days, and his findings are confirmed in our own material. In a series of 10 litter mates painted at weekly intervals from the fourth postnatal day onwards, the treated area in all the animals was completely epilated after 21, 44, and 65 days; that is, at intervals of 21 to 23 days.

Warts first appeared after 48 days, and were present in most mice after 60 days. The first tumor, histologically confirmed, was excised on the 70th day. Ulcerations in the treated area were seen in the later periods of the experiments; they healed rather quickly in spite of continued painting except for those in or around fairly large tumors.

Dermal changes, in the form of an inflammatory reaction, start in the deeper dermal layers, spread towards the subepidermal zone, and may involve the epidermis. Renewed paintings tend to exacerbate the inflammatory reaction until it reaches an almost constant level after about 5 weeks. The collagenous fibers, particularly of the subepidermal zone, are dissolved and replaced first by fibroblasts and from the fifth week onwards by new, thin-fibered collagenous tissue. At about this time the elastic fibers of the dermis begin to show some irregularities in arrangement and distribution. In some places there are only few and granular fibers, while in others there are more fibers which, however, tend to clump together. These changes are probably secondary to (a) the effect of the inflammatory reaction on the collagenous fibers, the arrangement of which determines to some extent the arrangement of the elastic fibers, and (b) the cycle of epilatory and regenerative changes in the hair follicles around which the elastic fiber

meshes are anchored. With the development of malignant tumors a localized focal inflammatory reaction is superimposed on the primary, more generalized, and rather diffuse dermal reaction.

The epidermal reactions to the first painting have been described above. The subsequent changes consist in increased epidermal hyperplasia and hyperkeratosis, in similar changes in the epidermal appendages, and in the formation of papillomas and malignant tumors. Epidermal hyperplasia always precedes tumor formation, which occurs only in a hyperplastic epidermal region, or in hyperplastic hair follicles. Papillomas may become malignant growths, they may remain—at least for some considerable time—papillomas without malignant change, or they may regress. Malignant growths may also arise directly in hyperplastic regions without the previous formation of a wart.

The epidermal thickening that follows the first application of benzpyrene is maintained at about the same level by the subsequent paintings for the first 6 weeks. The epidermal hyperplasia is generalized over the whole treated area, but is greater in some regions than in others. The increase in cell size most noticeable during the first week tends to diminish during subsequent weeks, though many large and binucleate cells are always encountered. Among the dividing cells a number of abnormal mitotic figures is seen. Some variation in thickness of the most active epidermal region is caused by the periodical conversion of stratum granulosum into stratum corneum, which occurs on about the 14th, 28th, and 42nd day.

The cyclical changes in the hair coat consist of a hypertrophy of follicles followed by hyperkeratosis and loss of hair, and later by the regeneration of follicles and sebaceous glands and the formation of hairs. At least 2 complete cycles are usually observed during the first 6 weeks. During this initial period the number of hair follicles per unit length of skin and the process of hair formation are not substantially altered.

Warts usually begin to form about the seventh week, in definitely thickened epidermal areas and in hypertrophic hair follicles (Figs. 6, 7, 8). The further increase in thickness of epidermal areas leads to the formation of partly keratinized excrescences as well as to downward projections. Similarly the hypertrophic hair follicles enlarge still further, their number tends to increase, and only abortive hair formation is found. The central parts of the follicles keratinize in the same manner as the epidermis and they are thus transformed into epidermal interpapillary processes, which remain recognizable by the occasional presence of hairs or their rudiments, by the attachment of parts of normal or metaplastic sebaceous glands, and by the persistence of a central structure derived from the original lumen or mouth of the hair follicle and now filled with keratin (Figs. 7, 8, 9). This central structure is not found in downward projections of hyperplastic epidermal regions. Very frequently the neighboring areas of hypertrophic epidermis and hypertrophic follicles become confluent and form the center of a wart (Figs. 8, 9, 10). These formations are never sharply defined at their periphery, but taper out into less hypertrophic regions.

The form of the ensuing papilloma is determined by the localization of its most actively growing part. A relatively greater growth rate in this periphery, as compared with the center, produces an inversion of the central parts and the formation of a cyst-like lumen filled with keratin (Figs. 11, 13), while a relatively greater growth rate at the center produces a broad-based wart with a center projecting outwards (Figs. 9, 10). Small keratinized cysts result from single enlarged and keratinizing hair follicles (Figs. 11, 12).

There is no evidence of anaplastic changes in the cell layers of the papillomas, which differ only quantitatively from those of the hyperplastic epidermis. The direction of growth towards the stratum corneum, *i.e.*, in a vertical direction from the basal layer, remains unchanged. Lateral expansion of papillomas is a result of increased growth of adjacent hyperplastic regions and not of lateral migration of "papilloma" cells and their multiplication. Apart from a dissolution of the basement membrane at the tip of the epidermal projections, and the diffuse inflammatory reaction, the dermal tissue does not seem to be involved in this purely epithelial hypertrophy.

Malignant foci appear as early as 10 weeks after the first painting in some mice. They arise in a hyperplastic area of the epidermis (Fig. 12), or more frequently in the growing parts of papillomas (Fig. 13). In either case the malignant change takes place in the basal layer, *i.e.*, among the resting cells, while at first the more differentiated layers remain unaltered. The induction of malignancy appears to be a gradual process, since it is impossible to find a clear demarcation line between malignant cells and their nonmalignant neighbors. Even fairly small foci are not sharply outlined. The malignant change may take place in the walls of keratinizing cysts and here, as in papillomas, the older parts go on keratinizing without reflecting any sign of malignant change. In branching cysts or papillomas malignancy may be induced at first in one branch only.

The morphological characteristics of the malignant change consist of (a) cellular changes such as increase in volume of cytoplasm, nucleus, and nucleolus, (b) qualitatively and quantitatively abnormal differentiation, (c) invasive properties. The character of the malignant formations varies with the develop-

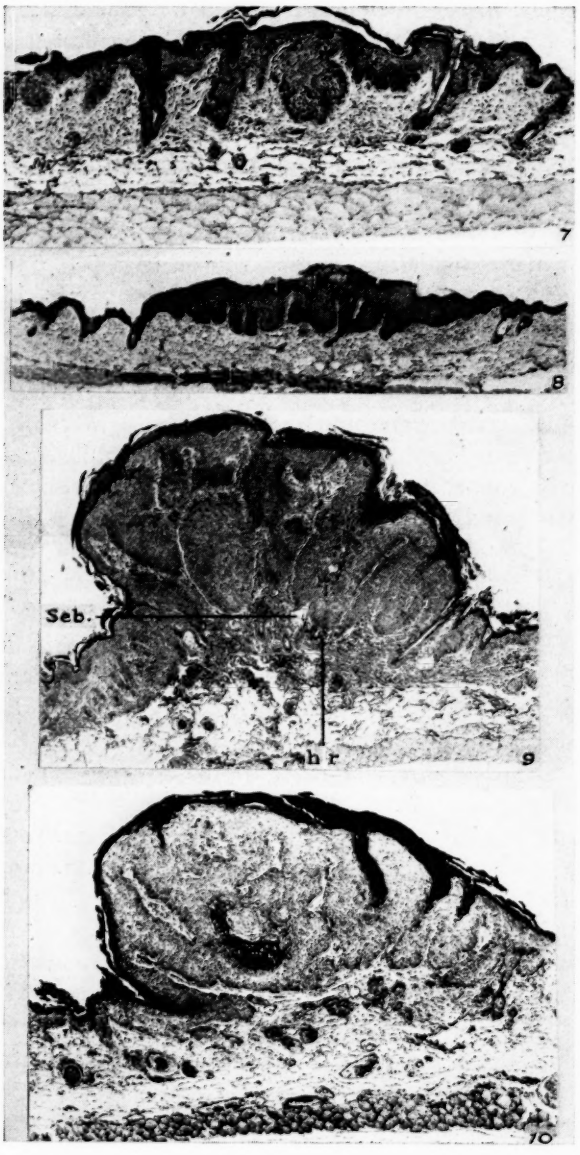

FIG. 7.—Mouse skin painted with benzpyrene at weekly intervals and fixed on 112th day of experiment. Hyperplastic epidermis forms a number of downward projections (interpapillary bodies). Hair follicles also hyperplastic and some show abnormal keratinization. (Other parts of same section shown in Figs. 11 and 12.) Mag. × 46.

FIG. 8.—Mouse skin painted with benzpyrene at weekly intervals and fixed on 70th day of experiment. The center of a wart is formed by a number of adjacent hyperplastic hair follicles and interpapillary bodies. Mag. × 27.

FIG. 9.—Mouse skin painted with benzpyrene at weekly intervals and fixed on 104th day of experiment. A wart formed by neighboring interpapillary bodies and hyperplastic hair follicles. Latter recognized by attachment of sebaceous glands (Seb) and hair rudiments (hr) and by keratin configuration at their mouths. Here, as in Figs. 6, 7, and 8, downward projections of epidermis appear as separate units and show same direction of growth and keratinization as normal epidermal structures. Mag. × 46.

FIG. 10.—Mouse skin painted with benzpyrene at weekly intervals and fixed on 84th day of experiment. A wart showing secondary fusion of hyperplastic hair follicles and of interpapillary bodies. Mag. × 35.

FIG. 11.—Same section as shown in Fig. 7. Note downward growth of papillomatous formations next to branching keratinized cyst. Hair follicles at periphery of cyst are hyperplastic and show abortive attempts at hair formation. Mag. × 26.5.

FIG. 12.—Same section as shown in Figs. 7 and 11. On left, a small invasive, anaplastic focus arises in the hyperplastic epidermis. On right, abnormal hair follicles and interpapillary bodies. Figs. 7, 11, and 12 illustrate the difference in degree of generalized reaction of mouse skin to benzpyrene painting. Mag. × 51.5.

FIG. 13.—Same section as in Fig. 10. Anaplastic invasive extensions growing from papillomatous region into dermis and into wall of keratinized cyst. The originally separate downward projections of epidermis fuse secondarily. Mag. × 26.

FIG. 14.—Mouse skin painted with benzpyrene at weekly intervals and fixed on 93rd day of experiment. On left, a keratinizing squamous cell carcinoma that invades panniculus carnosus. On right, a wart, joined by hyperplastic epidermal region to the carcinoma. Note localized inflammatory reaction induced by carcinoma. Mag. × 14.5.

mental stage and environment of the focus. Young foci are recognized by notable mitotic activity, by a proportionately large number of resting cells, and by a correspondingly small number of differentiating cells, which may proceed to normal keratinization in regular layers. More frequently the layer formation is disturbed and instead of normal keratinization cells undergo a parakeratotic change, *i.e.,* they form dense, almost hyaline material without having passed through the stage of forming keratohyaline granules, and they retain a pyknotic nucleus. Old foci consist of parakeratotic cells or keratinized debris, and are usually infiltrated by round cells.

The invasive properties of young malignant foci are due to their high mitotic rate, combined with histiolytic powers and their ability to evoke a localized inflammatory reaction. The growing malignant focus shares with any growing epithelial formation the ability to dissolve the basement membrane at the growing tip. It remains rather doubtful how much the malignant focus owes its invasive action to the presence of intracellular histiolytic agents, and how much to the effect of the inflammatory reaction. But whether the mechanism of tissue dissolution is direct or indirect, the invasive power is undoubtedly a new and even the final sign of the malignant change, and the invasion of dermal muscles is usually taken as the criterion of epidermal carcinoma in the mouse.

Malignant foci may appear simultaneously or at short intervals in the treated area at varying distances from each other; they frequently become confluent and form a single large tumor. Warts may persist unchanged for some time close to malignant foci (Fig. 14). The developmental stage of the foci, the different types and degrees of anaplasia, the nutritional conditions, and the stroma reaction contribute to the great variety in the appearance of tumors even in the same animal. For our quantitative analysis, which cannot demonstrate the anaplastic change or the acquisition of invasive properties, the youngest and most active tumor parts are chosen.

Cell counts made up to and including the stage of the formation of small papillomas refer to the same unit of skin as used in the other experiments. For the carcinomas the cell counts cannot be related to such a unit, and the results for this series are therefore expressed as percentage cell counts. In Chart VI the total cell count per epidermal unit before carcinoma formation is also given. The counts refer to epidermal hyperplasia up to day 49, to papillomatous regions from day 49 to day 70, inclusive, and to carcinomatous foci afterwards.

The initial quantitative changes are described above (first week in Chart V), and they are maintained with fluctuations around the same level for the first 6 weeks. The 3 troughs in the total cell count (Chart VI) are a result mainly of the conversion of stratum granulosum into stratum corneum. The period of papilloma formation is characterized by an absolute and relative increase in the number of resting cells, while the number of differentiating cells is reduced only relatively. Mitotic and degenerate cell percentages are not greatly altered during this period.

A further absolute and relative increase in number of the now anaplastic resting cells marks the period of malignant change. There is a further relative decrease in the number of differentiating cells, while

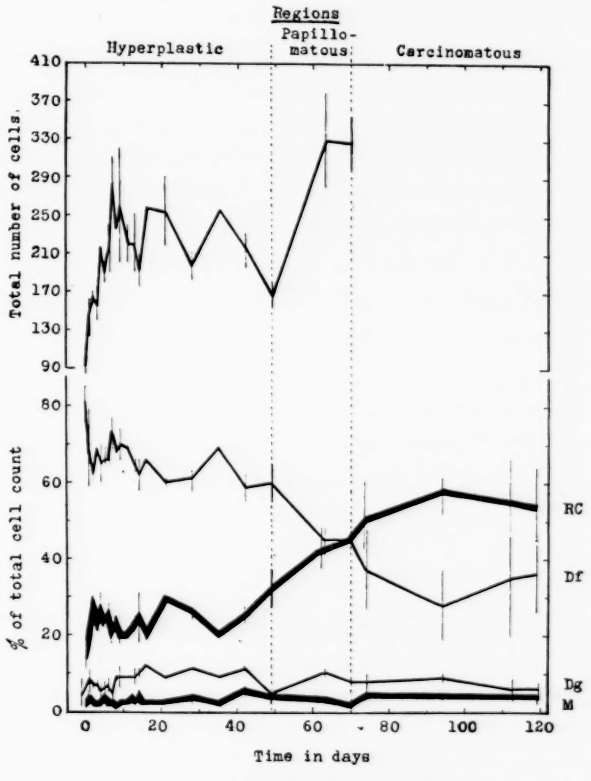

CHART VI

the mitotic and degenerate cell percentages persist on roughly the same high level. In the absence of a progressive change in percentage of mitotic and degenerate cells, papillomas and carcinomas are formed by the absolutely and relatively increased number of resting cells; this fact indicates that the "resting" phase is prolonged; *i.e.,* fewer resting cells become differentiating cells at any given time and the onset of differentiation is delayed (see below). The disturbance of the differentiation processes is shown qualitatively in the abortive hair formation; the scattering of sebaceous gland cells in epidermal regions; the increased number of epidermal interpapillary bodies (twice the number of normal hair follicles in the same unit of skin, Fig. 8); their increase in diameter (amounting to 2 or 3 times that of the normal hair follicle); and in the irregular keratinization of foci (parakeratosis and absence of proper stratification).

The reaction of mouse epidermis to repeated benzpyrene paintings may be summed up as an early and maintained rise in mitotic activity, followed by an absolute and relative increase in the number of resting cells, which, combined with a progressive relative decrease in the number of differentiating cells, leads to the formation of papillomas and later of carcinomas. The malignant change of resting cells occurs in hyperplastic epidermal regions, in hyperplastic hair follicles, or in papillomas. The differentiation process, previously disturbed only quantitatively, now undergoes qualitative changes. The early rise in number of degenerate cells is maintained, but does not interfere with the growth of the active areas.

(*c*) *The reaction of rat skin to single and repeated applications.*—The findings to be described under this heading are mainly negative, since the epidermis fails to react to the application of benzpyrene. There is no epilation, epidermal hyperplasia, or tumor formation. The only noticeable effect consists in a slight dermal infiltration immediately after application of the solution, indicating that the absence of an epidermal reaction is not referable to a failure of the solution to penetrate the thicker keratin layers of the rat's skin. Equally thick layers of keratin in treated or very young mouse skin fail to interfere with the penetration and action of the benzpyrene solution.

TABLE I: SHOWING AVERAGE CELL COUNTS WITH STANDARD DEVIATIONS FOR TREATED AND UNTREATED MOUSE EPIDERMIS

Group	Differentiating cells	Mitotic cells	Resting cells	Degenerate cells	Total cell count
1. Adult controls	92 ± 7.1	1.3 ± 0.22	16 ± 1.3	5 ± 1.3	114 ± 7.9
2. Acetone-painted mice	82 ± 5.9	1 ± 0.3	15 ± 1.4	4 ± 0.5	102 ± 5.9
3. Chemically epilated mice	149 ± 14.2	1 ± 0.4	23 ± 3.7	6 ± 1.3	180 ± 18
4. Turpentine-painted mice	134 ± 15	2 ± 0.35	36 ± 2.6	13 ± 3.8	185 ± 20
5. Benzpyrene-painted mice (single application)					
(a) 0.5 to 24 hours after painting	99 ± 4.2	3 ± 0.44	25 ± 1.5	10 ± 0.9	136 ± 5.5
(b) 2 to 7 days after painting	137 ± 11.5	5 ± 0.62	49 ± 2.7	12 ± 1.3	203 ± 13.5
(c) 11 to 63 days after painting	133 ± 11.1	2.4 ± 0.93	32 ± 3.6	6.6 ± 0.5	174 ± 14.2

DISCUSSION

The specificity of the initial epidermal hyperplasia.—For a convenient comparison of the quantitative changes elicited in the same unit of mouse skin by the various treatments, the average cell counts with their standard deviations are given in Table I. The table, in conjunction with the charts, shows that the same degree of epidermal thickening (see Groups 3, 4, and 5b in Table I) can be brought about in 3 ways: (a) by cellular migration from the hair follicles without any rise in epidermal mitotic activity (Group 3), a process that also deals adequately with the repair of minor trauma and burns (1); (b) by cellular migration supplemented later by increased cellular proliferation in the epidermis (Group 4, Table I). This process is also observed in the repair of larger wounds and burns; (c) by a definite and immediate increase in epidermal mitotic activity while the hair follicles enlarge and keratinize (Group 5).

The epidermal hyperplasia following benzpyrene painting is brought about differently from the usual type of regenerative hyperplasia. Benzpyrene hyperplasia is a result of increased mitotic activity and an absolute and relative increase in the number of resting cells. In regenerative hyperplasia the epidermal thickening is correlated in extent and duration with the extent of tissue loss. In benzpyrene hyperplasia the epidermal thickening following a single application in a nonirritant solvent is not related to any noteworthy degenerative effect and lasts for considerable periods. Finally, benzpyrene hyperplasia is specific, in that it is absent in animals of a nonsensitive species (rats), and is not elicited in sensitive animals by painting with noncarcinogenic hydrocarbons (57, 58).

Primary effects of benzpyrene on mouse skin.—The local application of benzpyrene in a nonirritant solution causes no significant cellular degeneration, nor has it the specific growth inhibitory effect that follows injection of carcinogenic hydrocarbons (31, 39). The toxic effect of hydrocarbons on mouse skin varies with the compound, its concentration, dose, and solvent, and also with the region (15, 17-20, 64) to which it is applied. But the absence of definite toxic effects in our own experiments confirms previous reports (15, 57, 58, 64), and shows that if such deleterious effects occur under other experimental conditions they are probably without significance for the subsequent tumor formation.

The stratification of the thickened mouse epidermis is an outcome of an increase in the number of cells but is not necessarily equivalent to increased differentiation, that is, to a relative increase in the number of differentiating cells. The epidermal hyperplasia following benzpyrene painting is due to (a) increased mitotic activity of resting epidermal cells (Table II), and (b) a delay in the onset of differentiation; *i.e.*, a relative prolongation of the resting phase (Charts V and VI, Tables I and II). These effects are followed during the final period of tumor

formation by interference with the differentiation processes.

The regular cyclical conversion of stratum granulosum into stratum corneum in benzpyrene hyperplasia, together with the regular period of the hair cycle (about 21 days) indicates that the duration of keratinization is not greatly altered during the early periods up to the appearance of warts. The *relative* increase in the number of resting cells and the relative decrease in the number of differentiating cells during this period indicate a prolongation of the resting phase by about 2 days (average duration of keratinization = 21 days, average percentage of resting cells = 14 per cent, average duration of resting phase = 3 days: with an average of 24 per cent of resting cells in benzpyrene hyperplasia the duration of the resting phase is increased to about 5 days).

differentiation impulses as evidenced by the prolongation of the resting phase, and might result finally in their anaplastic change. This reaction in mouse epidermis is generalized over the whole treated area, though varying in degree in different fields. There is no evidence that these changes result from the rapid proliferation of single cells. Though cellular as well as nuclear and nucleolar and chromosomal changes are observed, initially keratinization proceeds normally (except quantitatively) and both the epidermal hyperplasia and the warts are of a reversible nature. Most of these cellular changes are presumably lethal (5, 9), and the significance of the nonlethal cytological abnormalities remains obscure.

The delay in onset and the subsequent change in character of the differentiation processes may be secondary to the growth-stimulating effects of the

TABLE II: SHOWING THE AVERAGE NUMBER OF CELLS WITH STANDARD DEVIATIONS IN UNTREATED AND BENZPYRENE-PAINTED MOUSE EPIDERMIS

Group	No. of cells			Resting cells in mitosis, (per cent)
	Differentiating	Degenerate	Resting	Mitotic
1. Adult controls	92 ± 7.1	5 ± 1.3	16 ± 1.3	1.3 ± 0.2	8 (6-10)*
2. 0-24 Hours after single benzpyrene painting	99 ± 4.2	10 ± 0.9	25 ± 1.5	3 ± 0.4	12 (7-15)
3. 2-7 Days after single benzpyrene painting	137 ± 11.5	12 ± 1.3	49 ± 2.7	5 ± 0.6	10 (8-12)
4. 8-49 Days after repeated benzpyrene paintings	142 ± 7.6	21 ± 1.4	53 ± 2.3	6 ± 0.6	11 (10-13)
5. Papillomatous regions, 63-70 days after repeated paintings	148 ± 15.1	28 ± 4.2	143 ± 6.2	9 ± 2.0	6 (5-8)
6. Carcinomatous regions, 74-119 days after repeated paintings	83 ± 8.7	17 ± 1.8	132 ± 4.5	10 ± 0.9	8 (7-9)

* Ranges of variation.

Prolongation of the resting phase, which makes more cells available for division at a given time, does not account entirely for the increased number of mitotic cells in the painted skin, as the percentage of resting cells in division (Table II) is significantly increased during the period of epidermal hyperplasia, and particularly during the first day. Thus the epidermal thickening is due to both prolongation of the resting phase and more rapid proliferation of the cells. During wart and tumor formation the mitotic index in resting cells does not differ significantly from that of the controls. This finding agrees well with direct observations on the duration of the intermitotic period and of mitosis. The duration of the intermitotic period is of the same order for normal and malignant cells *in vitro* (40), while the mitosis of malignant cells tends to be slightly protracted (41).

The primary growth-stimulating effects of carcinogenic hydrocarbons consist of an increased mitotic activity and an increase in volume of the cells and their components (5, 16, 25, 54). Such active growth of cells might render them relatively insensitive to

carcinogens or may be interpreted as a separate direct effect on the factors determining the differentiation of mouse skin or on the response to them of the epidermal cells. The existence of such determining or "organizing" factors in mouse skin, and their variation with region, have been established in skin grafting experiments between mice of genetically pure lines (60-62). The location of epidermal cells in a skin region has been found to determine the differentiation of the cells or their descendants.

The evidence for such a direct, separate effect of carcinogens on differentiation in mouse epidermis is not conclusive, although there is a striking correlation between regional variation in sensitivity and regional variation in epidermal structure. The sensitivity to carcinogens decreases in a caudal and abdominal direction towards the pads of the feet (68, 70), and it is interesting that the thickness of epidermal layering and size of hairs increases, while the density of the hair coat decreases, in the same direction. A similar correlation may hold true for different strains of mice (7, 33, 34) and for different species, where size of hairs increases with decreasing sensi-

tivity to carcinogens and decreasing density of the hair coat in the order: mice, rabbits, rats, guinea pigs. This correlation of epidermal structure with sensitivity to carcinogens may be explained on the basis of a specific correlation of carcinogens with the differentiation process. A more likely explanation, however, lies in the fact that with reduced epidermal layering—which is always correlated with increased density of pelage—the hair follicles act as germinative centers for the epidermis, and that both react directly to the growth-stimulating action of the carcinogens. This action may be facilitated by the reduction of keratin layers in the sensitive regions of mouse skin. In rat epidermis no evidence of a growth-stimulating action by benzpyrene has been detected. On subcutaneous injections of carcinogens, however, sarcomas are elicited in the rat as well as in the mouse (27). Thus a specific sensitivity of mouse epidermis to the growth-stimulating effect of the carcinogenic hydrocarbons must be assumed.

The continuity of the carcinogenic process.—Though carcinomas may be elicited by a single application of carcinogenic hydrocarbons (19, 37, 46), in most animals repeated paintings are required to produce the same result. Thus of 11 litter mates subjected to 8 benzpyrene paintings at weekly intervals, 4 mice produced tumors and 2 more merely warts. In all these animals specimens taken from the painted area for biopsy show an initial epidermal hyperplasia. Animals dying 9 to 12 months later showed only slight or no epidermal thickening. The warts were observed for 4 months after their first appearance and during that period did not undergo malignant change. In other mice warts were seen to regress. Nonspecific stimulation of the hyperplastic or papillomatous regions, on the other hand, may induce the malignant change. There is, furthermore, no correlation between the rapidity with which warts appear in painted animals and the onset of malignant change (7).

All these findings indicate that, while in some mice the process of carcinogenesis may be continuous, in most animals the induction of malignancy represents a definitely new step in the process. The malignant change is not concerned primarily with the rate of cell proliferation, since neither the mitotic index (6) (see Table II) nor the growth rate is necessarily different from those of benign warts (47-49). Anaplastic changes in the cells are seen as quantitative and qualitative insufficiency and change of differentiation potentialities, and as the acquisition of invasive properties, including the induction of localized inflammatory reaction.

Whether these changes are secondary to further growth-stimulation by specific or nonspecific stimuli, or are the outcome of an entirely separate effect such as the chance mutation of a single cell, is not yet settled. In the former case we must assume that further stimulation of growth would render the cells unable to differentiate properly and lead to their anaplasia, and that this would involve changes in their genetic structure since the anaplasia is inherited by the descendants of these cells. These disturbances in cellular physiology would be paralleled by the nuclear abnormalities and the changes in volume and quantitative relationships of nucleus, nucleolus, chromosomes, and cytoplasm. In favor of such a conception is the occurrence of numerous simultaneous confluent malignant foci in the same animal (Figs. 7, 11, 12, 13, 14) or in the same patient (69).

In the latter case one of many somatic mutations must be assumed to succeed in the malignant conversion of a single cell, the descendants of which would overgrow the precancerous and papillomatous region. Here again the observed abnormalities in nuclear and cellular structure would serve as evidence of a labile state, and would thus appear as the symptom rather than the cause of malignancy (9). Such a possibility cannot be excluded. It should be emphasized, however, that it is in the final stage of carcinogenesis that an irreversible mutation must be assumed. The reversibility of warts and their relationship to the generalized epidermal hyperplasia render the assumption of early (48) or of successive mutational (12) changes unlikely.

SUMMARY

A quantitative histological analysis is given of

(a) The normal postnatal development of mouse epidermis.

(b) The regenerative hyperplasia induced in mouse skin by chemical depilation and by turpentine painting.

(c) The changes following the single and repeated application of benzpyrene to mouse and rat skin.

The development of the first pelage in the mouse is correlated with a reduction of epidermal layering. The hair follicles subsequently act as germinative centers for the epidermis. Stratification of the adult epidermis is not equivalent to differentiation.

Regenerative hyperplasia following chemical depilation is due to cellular migration from, and greater mitotic activity in, the hair follicles, and does not involve changes in epidermal mitotic activity.

Regenerative hyperplasia following repeated turpentine paintings is attributable at first to cellular migration and greater mitotic activity in the hair follicles, and subsequently also to increased proliferation of epidermal cells.

The initial hyperplasia of the epidermis and its appendages following benzpyrene painting is a result of a direct increase of mitotic activity in the epidermis and the hair follicles. These proliferative

changes are not secondary to primary deleterious effects. Benzpyrene in acetone solution applied to mouse skin causes a slight increase in the number of degenerate cells, but does not lead to early ulceration or to a specific inhibition of growth. Epilation is secondary to increased proliferative activity in hair follicles and hair sheaths.

The primary reactions of mouse epidermis to benzpyrene painting are (a) increase in cell growth (both number and size of cells); (b) delay in the onset of differentiation. These reactions are generalized over the whole treated area, and result in an absolute and relative increase in the number of resting cells. These changes account for the hyperplasia of the epidermis and its appendages and for the formation of papillomas.

Carcinomas develop in hyperplastic or in papillomatous regions. Their appearance is characterized by a further absolute and relative increase of the now anaplastic resting cells, by a quantitative and qualitative disturbance of the differentiation process, and by the induction of a focal inflammatory reaction. The mitotic index of carcinomas may not differ from that of nonmalignant formations.

The bearing of these findings on the interpretation of carcinogenesis in mouse epidermis is discussed.

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The Disappearance of Carcinogenic Hydrocarbons in Autoxidizing Lipids*

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Previous investigations from this laboratory have demonstrated that carcinogenic chemicals inhibit the autoxidation of aldehydes and lipoidal materials (4, 5, 15, 19). The presence of 3,4-benzpyrene in these oxidizing media resulted in the production of brown substances that were not attributable to derivatives of the lipid, and presumably originated in the oxidative degradation of the hydocarbon. Studies have now been made on the rate of disappearance of certain carcinogenic hydrocarbons dissolved in lipids of varying degrees of autoxidizability. In addition, two colored compounds were identified in autoxidized lipid mixtures containing 3,4-benzpyrene.

METHODS

Standard lipid samples containing the carcinogens were prepared by transferring known quantities of these materials, dissolved in acetone or Skelly solve B,[1] to individual test tubes. In certain tubes 200 μgm. of α,β-tocopherols were added in the same manner. After all additions the volatile solvent was removed by evaporation in a vacuum desiccator. A typical test sample contained 5 mgm. of lipid and 50 μgm. of hydrocarbon. The mixtures were then allowed to autoxidize in air at room temperature in the absence of light. At intervals duplicate samples were diluted with redistilled acetone to the proper hydrocarbon concentration, and analyzed fluorometrically for remaining hydrocarbon (12). No fluorescence was noted in the diluted lipid samples alone during the experiments, nor did the presence of lipid, fresh or oxidized, affect the fluorescence of the hydrocarbons. Hence the difference between the analyses at zero time and after a given period of autoxidation was considered to represent the amount of hydrocarbon destroyed.

The ethyl linolate was prepared by the method of Rollett (14) and further purified by distillation *in vacuo*, and the linoleic acid was obtained by the hydrolysis of this ester. The acid was a colorless liquid with an iodine number of 178. Cottonseed oil was largely freed from antioxidants by passing a solution of the edible commercial oil in Skelly solve B through separate columns of activated alumina and neutrol-5 [2] (17). Final purification was effected by molecular distillation. Mouse fat was extracted from minced eviscerated mouse carcasses with Skelly solve B in a Waring blendor; the crude viscous oil remaining after evaporation of the solvent *in vacuo* was used for these experiments. A lard filtrate was obtained by the filtration of fresh commercial leaf lard through qualitative filter paper at 38° C. The semi-solid material remaining on the filter is termed the lard residue (10). The tocopherol sample was a purified mixture of α and β tocopherols obtained from Merck and Co. The carcinogenic hydrocarbons 3,4-benzpyrene, 20-methylcholanthrene, and 1,2,5,6-dibenzanthracene were Hoffmann-LaRoche products.

RESULTS

When 3,4-benzpyrene was added to linoleic acid and the mixture allowed to autoxidize at room temperature it gradually became golden brown. Analysis of the samples at intervals throughout the period of autoxidation demonstrated a progressive destruction of the carcinogen. In a typical experiment 35 per cent of the benzpyrene was destroyed during the first week, and 74 per cent in 8 weeks (Table I). The addition of 200 μgm. of α,β-tocopherol to similar samples delayed the destruction of benzpyrene. Thus during the first 4 weeks only 1 per cent of the carcinogen was destroyed, as compared to 66 per cent in the sample without tocopherol. However, after this latent period a rapid disappearance of the hydrocarbon was observed, which, after 8 weeks, approached that

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[1] A commercially obtainable mixture of hexanes, b.p. 66 to 68° C.

[2] Obtained from the Filtrol Corporation, Los Angeles, California.

occurring in the control samples. Benzpyrene was also destroyed in autoxidizing ethyl linolate, but at a slower rate than when dissolved in linoleic acid. In this mixture the addition of tocopherol also decreased the rate of destruction, but the effect was much less pronounced than in the experiment with linoleic acid. These observations are compatible with the slower autoxidative rate of ethyl linolate as compared to the free acid. When ethyl oleate was used as the solvent the disappearance of benzpyrene occurred at a still slower rate. Thus only 15 per cent of the hydrocarbon was destroyed after 8 weeks of autoxidation, and the addition of tocopherol had only a slight influence on the rate of destruction.

For the separation of the colored compounds formed in oxidized mixtures of linoleic acid and benzpyrene it was necessary to work with larger quantities. Mixtures of 5 ml. of pure linoleic acid and 5 mgm. of benzpyrene were allowed to autoxidize in petri dishes at room temperature for 4 to 7 days. Considerable color developed during this period, without any obvious changes in the physical properties of the linoleic acid. The mixture was dissolved in benzene and adsorbed on 0.7×50 cm. columns of activated 100 mesh aluminum oxide. The columns were standardized with a mixture of the pure 5,8- and 5,10-quinones of benzpyrene obtained by chromic acid oxidation (18). The presence of linoleic acid interfered to some extent

TABLE I: THE DISAPPEARANCE OF CARCINOGENIC HYDROCARBONS IN AUTOXIDIZING LIPIDS

(All figures are averages of duplicate readings)

Hydrocarbon, 50 μgm.	Lipid, 5 mgm.	α,β-Tocopherols, 200 μgm.	Per cent hydrocarbon destroyed by 1 week	2 weeks	4 weeks	6 weeks	8 weeks
3,4-Benzpyrene	Linoleic acid	—	35.0	54.0	66.0	69.0	74.0
"	" "	+	0.6	0.6	1.0	44.0	67.0
"	Ethyl linolate	—	7.0	16.0	46.0	50.0	72.0
"	" "	+	0.0	5.1	37.4	49.0	72.4
"	Ethyl oleate	—	0.0	7.5	11.0	9.0	15.5
"	" "	+	0.0	3.4	7.7	7.2	10.0
"	Cottonseed oil (chromatographed)	—	0.0	0.0	7.7	7.2	10.0
"	Mouse carcass fat	—	—	—	—	—	—
"	Lard filtrate	—	0.0	0.0	0.0	5.1	9.8
"	Lard residue	—	0.0	2.8	5.7	4.5	6.6
"	Tricaprylin	—	0.0	2.4	8.0	7.8	7.4
20-Methyl-cholanthrene	Linoleic acid	—	84.0	91.8	92.2	93.5	94.0
1,2,5,6-Dibenz-anthracene	" "	—	0.0	0.0	0.0	1.2	0.7

When chromatographed cottonseed oil was employed as the solvent, the rate of disappearance of 3,4-benzpyrene was relatively slow, so that after 8 weeks only 22 per cent had disappeared. Similarly, only 10 per cent of the hydrocarbon was destroyed when dissolved in fat obtained from the mouse carcasses. The disappearance of benzpyrene was even less rapid when tricaprylin, lard filtrate, and lard residue were employed as solvents. When these lipids were used, only 5 to 7 per cent of the carcinogen disappeared during the period of oxidation (Table I).

20-Methylcholanthrene was found to be more rapidly destroyed than 3,4-benzpyrene in oxidizing linoleic acid while 1,2,5,6-dibenzanthracene proved to be relatively stable in this medium (Table I). Eighty-four per cent of the methylcholanthrene disappeared in the first week of autoxidation and the originally colorless mixture became light green. The high stability of dibenzanthracene in this experiment agrees with its strong resistance to attack by chemical and physical agents (6, 11).

with the adsorption of the colored substances by the activated alumina; nevertheless a distinct separation of the compounds was obtained by repeating the chromatographing procedure 3 times. The third adsorption of the oxidized mixture separated the colored substances into an upper red band, which faded into a lower yellow band. Homogeneous sections of these bands were removed and eluted with acetone. The identities of these compounds were established through their adsorption characteristics and absorption spectra. Mixtures of each compound with the corresponding authentic quinone in benzene were adsorbed on columns of alumina, and found to yield single bands that gave no evidence of separation after traversing a distance of 40 cm. The absorption spectra of the acetone eluates were determined with a Cenco-Sheard spectrophotelometer. An absorption maximum of approximately 460 mμ. was observed with the red compound, while the yellow derivative exhibited maxima at approximately 430 and 450 mμ. The spectra of the known quinones corresponded closely to the curves ob-

tained with the autoxidation derivatives. Thus it appears that in the presence of oxidizing linoleic acid benzpyrene is oxidized to the 2 known quinones. At any instant the quantity of quinones that could be isolated from such mixtures represented only a small portion of the amount of hydrocarbon found to have been destroyed. Futhermore, considerable dark unidentified material, which was derived from the hydrocarbon, was found adsorbed at the top of the chromatograph columns. Since the quinones also have been found to be rapidly destroyed in the presence of oxidizing linoleic acid (13), these substances are probably derived from the first stages in the oxidative destruction of benzpyrene.

DISCUSSION

The experiments above demonstrate that both 3,4-benzpyrene and 20-methylcholanthrene are destroyed when present in autoxidizing fats. In the case of benzpyrene, there is a coincident formation of 3,4-benzpyrene-5,8-quinone and 3,4-benzpyrene-5,10-quinone, plus unidentified substances. Apparently these oxidation products are the result of a coupled oxidation similar to that taking place when carotene (16), *p*-dimethylaminoazobenzene (7), or hemin (9) are destroyed in the presence of oxidizing fats. Part of the destruction of hydrocarbon that occurs after application to tissues may be due to a similar oxidation occurring in the vehicle employed or in the tissue lipids themselves.

Berenblum, Chalmers, and their associates (1, 2, 3) have shown that 3,4-benzpyrene is oxidized *in vivo*, presumably to 8-hydroxy-3,4-benzpyrene, which is readily oxidized in air to 3,4-benzpyrene-5,8-quinone. These derivatives were isolated from the excreta of rats and mice injected with the hydrocarbon. Thus the oxidations of benzpyrene *in vivo* and *in vitro* have a common product and possibly a common point of attack. These oxidation products of benzpyrene have proved to be noncarcinogenic (2), and application of the intact hydrocarbon seems necessary for the production of tumors (8). Possibly the reactions that lead to the formation of these derivatives are involved in the carcinogenic process.

SUMMARY

The rates of destruction of 3,4-benzpyrene, 20-methylcholanthrene, and 1,2,5,6-dibenzanthracene were followed fluorometrically after these hydrocarbons had been added singly to various lipids and the mixtures exposed to air at room temperature for several weeks. Benzpyrene and methylcholanthrene were rapidly destroyed in oxidizing linoleic acid, and losses of 35 and 84 per cent respectively were noted after 1 week. 1,2,5,6-dibenzanthracene, on the other hand, was relatively stable to this oxidizing agent. Benzpyrene disappeared also, though less rapidly, in ethyl linolate, ethyl oleate and chromatographed cottonseed oil. The addition of tocopherol to certain of these mixtures delayed the onset of the destruction but did not alter the final result. Benzpyrene was relatively stable when dissolved in tricaprylin, lard fractions, or mouse carcass fat.

The isolation of 2 of the colored oxidation products of benzpyrene was accomplished by chromatographic adsorption of an autoxidized benzpyrene-linoleic acid mixture on activated alumina. These compounds were identified as 3,4-benzpyrene-5,8-quinone and 3,4-benzpyrene-5,10-quinone by mixed chromatographs with the authentic quinones and by their absorption spectra.

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On Tumor-Producing Chemical Substances*

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The following is a review of the studies carried out in this laboratory during the past seven or eight years with the view of (a) elucidating the effect of certain exogenous tumor-producing chemical agents and (b) ascertaining the presence within the human body of chemical substances that may call forth the formation of tumors. The present review is based in the main on the results obtained by the author and his associates; data from the literature will be only occasionally mentioned.

I. EXOGENOUS BLASTOMATOGENIC AGENTS

Virchow's conception of "irritation" as the cause of tumors has led to a long series of studies whose aim was to produce cancer experimentally through the agency of diverse stimuli. Observations on some kinds of occupational cancer in man, in particular on the skin cancer of chimney sweeps, pointed to the distillation products of coal, and an important advance was Yamagiwa and Itchikawa's discovery that cancer can be produced in laboratory animals by painting their skin with tar. The problem of tar cancer was taken up by numerous students so that we are now in a position to produce cancer systematically in a great number of cases; to study thoroughly its morphology and morphogenesis; to ascertain the presence of regularly appearing precancerous changes; and finally to raise a number of questions concerning pathogenesis and etiology.

At first the appearance of the tumor on the painted skin was regarded as conclusive evidence in support of Virchow's conception, as the growth was attributed to the "irritating" toxic action of tar, which leads to chronic inflammation, injury of the tissues, and their constant pathological regeneration. However, comparison of the carcinogenic action of different tars and their fractions with their chemical composition suggested that the tumor-producing capacity is appropriate not to the tar as a whole, but only to certain ingredients. According to Leitch (1922) coal tar may contain a "specific" cancer incitant in the form of definite chemical substances, and the long series of investigations by Twort, Bloch, Kennaway, and others contributed to an elucidation of the problem. Thus there arose the concept of carcinogenic substances, which at first was a mere hypothesis but through the discoveries of Kennaway, Hieger, Mayneord, Cook, and others later became an established fact. A new trend of study was inaugurated by the publication of the first data on cancer produced by means of 1,2,5,6-dibenzanthracene and other chemically pure substances, and a number of important facts have been brought forward within the past decade. Although these studies are a mere continuation of those dealing with tar, they have solved a number of problems in a way different from that which had been possible until then. It is only natural, therefore, that our investigation of carcinogenic tars, pursued during a number of years, should have culminated in a study of pure carcinogenic agents.

Substances Tested. Mode of Administration. Solvents

The following substances were used: (a) 1,2,5,6-dibenzanthracene, (b) 9,10-dimethyl-1,2-benzanthracene, (c) 9-methyl-10-ethyl-1,2-benzanthracene, (d) 3,4-benzpyrene, (e) 3,4,8,9-dibenzpyrene, (f) *o*-aminoazotoluene, and (g) *p*-dimethylaminoazobenzene, and others. All these were synthesized in the U. S. S. R., and some in this laboratory. A number of other compounds tested proved inactive. For skin applications benzene was generally used as solvent, and for subcutaneous administration olive, sunflower, or castor oil. Some experiments, however, showed that skin cancer may be obtained by painting the skin with the carcinogenic agent dissolved, not in benzene, but in sunflower oil. Again subcutaneous administration of the compound in crystalline form, without any solvent, likewise caused tumor formation. Apart from subcutaneous and intraperitoneal injection and skin painting, the carcinogens were sometimes administered with food or introduced as vaginal plugs (Klenitzky). Most of the experiments were carried out on mice, but in some rats, rabbits, or guinea pigs were employed. In the last named, large doses of 1,2,5,6-dibenzanthracene (25 to 48 mgm.) produced

* Because of the difficulties of international communication the author has not read proof of this article.

malignant growths after 19 to 22 months (Shabad), a confirmation of Haagensen and Krehbiel, who reported the induction of sarcoma in guinea pigs with 3,4-benzpyrene.

Malignant Tumors at the Site of Application

These tumors are distinguished by their multiformity. Squamous cell keratinizing carcinoma is the most common in mice after skin painting, but other types of cancer occur as well, such as squamous cell cancer without keratinization, spindle-cell carcinoma resembling sarcoma, and cancer originating from the sebaceous glands. Along with epithelial tumors, painting of the skin may incidentally result in the formation of sarcoma, presumably because the carcinogen penetrates through abrasions of the skin into the subcutaneous tissue.

At the site of subcutaneous administration there usually develop sarcomas, which may vary widely in structure. Besides the spindle cell type there occur polymorphous cell sarcomas and, somewhat less frequently, fibrosarcomas. As to their origin, some are derived from muscle: the malignant leiomyomas and the less common rhabdomyomas. The source of the latter is indicated by their pronounced polymorphism, their numerous large cells, and by traces of striation (Kleinenberg). In some cases the sarcomas of mice have been particularly rich in thin-walled, dilated vessels, which imparted to them the appearance of angiosarcoma. In the guinea pig a peculiar sarcoma of adipose tissue was discovered, *i.e.,* a malignant lipoblastoma, which metastasized to the lungs and eyes. Finally, in one case the subcutaneous administration of 9-methyl-10-ethyl-1,2-benzanthracene resulted in the formation of a malignant neurinoma.

Application of tar and dibenzpyrene plugs to the uterine cervix produced squamous cell carcinoma, either with keratinization or not.

Particular attention is directed to the fact that in a number of cases malignant tumors formed not directly at the site of administration, but close to it. Thus, after the subcutaneous injection of carcinogens a papilloma (Shabad) and a squamous cell carcinoma (Kleinenberg) appeared close to the injection sites, and the subcutaneous administration of 9,10-dimethyl-1,2-benzanthracene called forth, besides sarcoma, a mammary cancer. These were separate tumors, but in some cases such growths may fuse and present the picture of so-called carcinosarcoma.

Azo compounds elicited hepatomas in different stages of malignancy, and much less frequently tumors from the epithelium of the bile ducts, the so-called cholangiomas. It should be noted that multiple primary hepatomas occurred paralleling the great number of tumors produced in the skin and subcutaneous tissues by hydrocarbons. Azo compounds often caused peculiar proliferations of the stromal cells of the liver, producing infiltrates after the type of leukosis (Morozenskaya).

Thus carcinogenic compounds induce tumors not only at the site of application but also at a distance from it, and not only malignant tumors but most often benign tumors as well.

Transplantation of Induced Tumors

This was carried out in a number of cases, and five new sarcoma strains with one of liver cancer were carried through many generations. Microscopic examination of these growths showed that transplantation had not modified their general structure; even under the skin the liver cancer still retained certain features in the 67th generation characteristic of primary liver tumors (Morozenskaya).

Remote Tumors

Tar experiments have already shown that besides the tumors at the site of application a great number of different structures arise in remote regions. The possibility of producing remote neoplasms has been confirmed in this laboratory by numerous experiments with chemically pure blastomatogenic substances. Thus in mice treated subcutaneously, intraperitoneally, *per vaginam,* or by skin painting, multiple primary skin tumors were often noted in nontreated regions, which frequently originated from the sebaceous glands; as well as many primary adenomas of the lung and, somewhat less frequently, tumors of the mammary glands, cancer of the buccal mucosa, and lymphosarcoma of the thymus. A critical examination of these facts suggests the following doubts:

Are these remote tumors spontaneous? It is well known that tumors of the lung and mamma may spontaneously occur in mice and, although much less frequently, sebaceous adenoma and lymphosarcoma of the thymus. This objection can be refuted, however, since our mice belonged to a strain (RV) that has been under observation for about 14 years, during which the frequency of spontaneous growths has been an object of special study. Primary tumors of the lungs, for instance, occur in 5.2 per cent of cases, or in 8.5 per cent of mice above 8 months old; cancer of the mammary glands in 1.2 to 2.0 per cent of the females, and adenoma of the sebaceous glands only in isolated instances (Shabad and Kleinenberg). After the administration of blastomatogenic agents lung tumors were recorded in 22.5 to 36.2 per cent (application of 1,2,5,6-dibenzanthracene) or in 30 to 50 per cent (injection of 3,4,8,9-dibenzpyrene), etc.; *i.e.,* in a number of cases that is far beyond the possibility of spontaneous occurence.

If remote tumors are called forth by a carcinogen, does their localization depend on its direct penetration: into the lungs by the inhalation of particles adhering to the skin; into the mammary glands through their ducts; into distant skin areas by licking, scratching, etc.? The administration of blastomatogenic substances *per vaginam* showed that although in this case the dissemination of the substance to the skin was excluded, many remote tumors developed nevertheless in the skin. However, a decisive answer was obtained by injecting blastomatogenic substances under the skin and into the peritoneal cavity. In this case also there occurred cancer of the mamma, adenoma of the lungs, and sebaceous adenoma. For example, adenoma of the lungs followed the subcutaneous administration of 1,2,5,6-dibenzanthracene in 27 per cent of cases, or in 39 per cent of mice surviving 3 months after the beginning of the experiment.

Finally, comparison of the occurence of remote tumors with the strength of the blastomatogenic substance, as estimated by the number of tumors formed at the application site, conclusively showed that the remote tumors are likewise produced by the agent. Thus with a similar mode of treatment and equal amounts of administered substance, 3,4,8,9-dibenzpyrene caused a greater number of lung tumors than 1,2,5,6-dibenzanthracene, while the still more effective compound, 9,10-dimethyl-1,2-benzanthracene, produced adenoma of the lungs in 80 per cent of mice within 8 months after the beginning of the experiment (Kleinenberg). Again, upon the administration of the same substance in different doses the number of remote tumors increased with increase in the dose (Shabad and his associates).

Accordingly there is no doubt that the agents producing tumors at the site of application may also elicit neoplasms distant from it, in particular in some internal organs. Hence, one can judge the blastomatogenic properties of a compound also by the appearance of remote tumors, in particular, lung adenoma. Their occurrence may indicate, also, that blastomatogenic agents exert a general action upon the organism within which they circulate. Circulation of these compounds, conclusively demonstrated by the fluorescence method, was suggested by the observations on remote tumors. Finally, the appearance of these neoplasms makes it very probable that blastomatogenic substances may become localized beyond the site of their administration.

Systematic study of the blastomatogenic action of azo compounds (Morozenskaya) also showed that with different modes of administration a number of remote tumors occurred in addition to the hepatic neoplasms: lung adenoma (in 28.7 per cent of mice surviving 8 months), adenoma of the sebaceous glands, or mammary cancer. These facts, confirmed by Andervont, suggest that in spite of the specificity of azo compounds, which to a certain degree are organotropic with regard to the liver, one may find some common features in the blastomatogenic action of azo compounds and the carcinogenic hydrocarbons.

The appearance of remote tumors in connection with diverse blastomatogenic substances raises the problem of localization. Despite circulation of the blastomatogenic substances, the tumors occur only at definite, so to say favorite, places. This is presumably to be accounted for in part by the circulation paths of these substances within the organism. Thus the skin as well as the mammary glands, the sebaceous glands, and the lungs may be regarded as destinations for fats and lipoids, which may act as solvents of the blastomatogenic compounds. On the other hand, a certain significance is to be attached to the structure of the substance as well. This may well be exemplified by *o*-aminoazotoluene: Administration of this substance by mouth as well as its subcutaneous injection and application to the skin in benzene solution, invariably resulted in the appearance of liver tumors; whereas at the site of its administration, *e.g.*, in the painted skin, not only tumors but even initial changes were altogether absent (Morozenskaya).

Local Action of Blastomatogenic Compounds and the Role of Inflammation

Systematic inspection of the experimental animals and microscopic examination of the site of administration of the blastomatogenic substance, and of the tumors caused by it, showed that the severe acute and subsequent chronic inflammation so common with carcinogenic tars is absent. Indeed, the inflammatory changes in skin painted with 1,2,5,6-dibenzanthracene are no greater, but rather less pronounced, than with pure benzene (Shabad).

Subcutaneous administration of dibenzanthracene in vegetable oil results immediately in an acute inflammatory reaction, which, however, is not more active than that set up by the solvent alone. Several days thereafter the inflammatory reaction subsides and encapsulation of the droplet ensues, followed by partial resorption of the oil. The "oleoma" is gradually transformed into an "oleogranuloma", but the phenomena accompanying the administration of the oil with dibenzanthracene are quite similar to those following administration of the oil alone.

When oil was administered with dibenzanthracene or some other blastomatogenic substance one could note, 3 to 3½ months later, the beginning proliferation of richly cellular connective tissue in one or several areas of the oleoma wall; *i.e.*, the beginning of sarcoma. In no case did we note any signs of in-

flammation. The accumulations of proliferating and but slightly differentiated connective tissue cells were distinguished by their uniformity. No dilatation of the vessels or escape of blood elements or plasma was noted. Finally, there were no traces of maturation of the newly proliferating connective tissue, such as the abundant formation of collagen fibers, organization, shrinkage, etc. Thus neither during organization of the oleoma and formation of the oleogranuloma nor, and this is particularly important, at the moment of sarcoma formation, did the blastomatogenic substance cause any inflammatory changes, in particular inflammatory proliferation such as might precede tumor formation.

Special experiments were carried out in which 1,2, 5,6-dibenzanthracene was injected at laparotomy directly into the stomach wall in rabbits so that it lay beneath the mucosa. The solution did not cause any inflammatory changes except a slight natural reaction to the oil: at least no such active inflammatory proliferations of the epithelium as those readily called forth by the injection of tar into the stomach wall (Haga, Garshin).

Of particular interest is the microscopic examination of the site at which remote tumors arise. There is absent here even the inflammatory reaction that invariably occurs at the site of injection of any substance and hence always takes place in skin painted with blastomatogenic substances and at the site of their subcutaneous injection. It is safe to conclude from numerous data that remote tumors are not preceded by any ordinary or specific inflammatory changes, and that these play no role in their genesis.

In this connection we may mention the hepatic tumors caused by azo compounds. No early inflammatory changes are found in the liver, and the peculiar proliferations of the stroma cells noted at advanced stages are modifications of the leukosis type. In our numerous experiments on mice cirrhosis never occurred, though this is supposed to take part in the genesis of primary cancer of the liver in man.

Finally, special experiments were carried out to ascertain the role of acute and chronic inflammation in the genesis of skin cancer following the application of chemically pure blastomatogenic substances (Monastyrskaya). With 3,4,8,9-dibenzpyrene in vegetable oil inflammatory changes were found in the skin at an early date, but these were not more pronounced than those set up by an oily solution of 3,4,8,9-dibenzpyrene-quinone, which is not carcinogenic at all. A turpentine solution of 3,4,8,9-dibenzpyrene called forth more extensive inflammatory changes in the skin than the same amount of this substance in benzene, although this of course was to have been expected. Yet with the benzene solution there were not fewer, but more, tumors than with the turpentine solution.

It can not be denied that some blastomatogenic substances may exert, besides their carcinogenic action, a considerable local and general toxic effect. Thus, in guinea pigs subcutaneously treated with large doses of 1,2,5,6-dibenzanthracene there occurs a peculiar liver cirrhosis (Shabad and Urinson), while benzpyrene and dibenzpyrene, even in oily solution, cause more extensive inflammatory changes in the skin of mice than 1,2,5,6-dibenzanthracene. The most powerful blastomatogenic substance available in this laboratory, 9,10-dimethyl-1,2-benzanthracene, causes considerable degeneration and necrosis in the injected tissues. A correlation between this increased blastomatogenic effect and the increased tissue injury has not yet been established with certainty. It will be noted that some of the compounds tested, such as "sulfofresol" and "emulsol", caused a pronounced inflammation in the painted skin although their blastomatogenic effect was slight. Although final elucidation of the role of inflammation in carcinogenesis must be deferred to further investigation, it may be suggested that it is less significant than so far assumed; or at least that it may have a somewhat different significance. While prior to the discovery of chemically pure blastomatogenic substances some clinical data suggested that chronic inflammation itself may be a precancerous condition, the concept of precancerous change acquires at present a more restricted specific sense. If the effect of the blastomatogenic substance is not accomplished by way of inflammation it may be assumed that we are dealing here with a more specific, more delicate, and more intimate action upon the tissues, perhaps resembling that of the hormones, for example.

Correlation Between Chemical Structure and Blastomatogenic Action

The correlation between blastomatogenic action and chemical structure has already been extensively studied, principally by two groups: Kennaway, Cook, and others; and Fieser, Shear, and their associates. We can supplement their observation by several examples from this laboratory. Thus in the systematic study of a number of derivatives of 1,2-benzanthracene, synthesized by Mikhailov in the laboratory of Professor Ushakov and tested by Kleinenberg, it was found that 1,12-trimethylenechrysene is a very weak blastomatogenic substance. Subcutaneous administration never produced sarcoma at the site of injection. Application of an oily solution to the skin of mice throughout their life cycle finally elicited papillomas, which, however, were never transformed into cancer; in the internal organs, particularly in the lungs,

primary adenomas were detected in a comparatively large number of cases. It will be noted that in its chemical properties this substance may be regarded not only as a derivative of chrysene but of 1,2-benzanthracene as well.

The next stage in the systematic study of derivatives of 1,2-benzanthracene was the assay of 9,10-dimethyl-1,2-benzanthracene. This substance proved a very powerful blastomatogenic agent, in that it was capable of inducing sarcoma when administered in as small a dose as 0.1 mgm. Painting of the skin of mice with a 0.1 per cent solution in benzene (Prokofieva) produced papillomas as early as the 12th day of the experiment. According to Kleinenberg, painting with 0.05 per cent solution elicited papillomas in almost all experimental animals beginning at the 40th day, and cancer after 2 to 3 months. There also occurred especially numerous remote tumors, lung adenomas in particular.

9-Methyl-10-ethyl-1,2-benzanthracene likewise proved a very strong blastomatogenic agent (Kleinenberg), but it did not reveal the toxic and necrotic properties characteristic of 9,10-dimethyl-1,2-benzanthracene.

Another homologue of the same series was studied, in which the radical of acetic acid occupies position 10 while position 9 is occupied by the methyl group. The investigation of this substance (9-methyl-1,2-benzantryl-10-acetic acid) appeared of particular interest since it may be a preceding stage in the synthesis of 9,10-dimethyl-1,2-benzanthracene. It was found that while the last link in the whole chain of synthesis is a very powerful blastomatogenic agent, the link immediately preceding it is biologically inactive. Similar relations were found with regard to methylcholanthrene, which is preceded in the course of synthesis by inactive dehydronorcholene. Hence it may be concluded that the blastomatogenic properties of a substance appear suddenly in connection with its structure. It will be of interest to note that the acetic acid derivative is comparatively more akin to compounds that may occur in the organism than are other derivatives of 1,2-benzanthracene.

Experiments with 3,4,8,9-dibenzpyrene showed that this is a strong blastomatogenic agent. Upon subcutaneous administration it produces sarcoma in nearly 100 per cent of cases, and applied to the skin it elicits papillomas in almost all mice and cancer in two-thirds of them. Moreover, there were (Kleinenberg) multiple lung tumors (30 to 50 per cent), cancer of the mammary glands (in one-third of the females), and other remote tumors such, for example, as lymphosarcoma of the thymus. On the other hand, 5,10-quinone-3,4,8,9-dibenzpyrene had no blastomatogenic activity at all. Of particular interest is the fact that the introduction of oxygen into the molecule of a carcinogenic substance deprives it of the capacity to produce tumors.

Our observations, as well as those of others, testify that modification of structure affects blastomatogenic properties. In our view, the facts citied above suggest that modifications of structure such as might occur within the organism may neutralize carcinogenic action; *i.e.,* may act as antiblastomatogenic factors. In this connection we may cite another observation made in this laboratory on solutions of 9,10-dimethyl-1,2-benzanthracene. It was found that when this compound was exposed to light and air for 2 or 3 months it showed an appreciable decrease in carcinogenic activity. Hence it may be assumed that the oxidation of blastomatogenic substances may play the role of an antiblastomatogenic factor.[1]

Antigenic Properties of Blastomatogenic Substances

Our efforts to control the development and growth of neoplasms have included the treatment of transplanted tumors with spleen extracts, spleen itself, and blood. It was shown on extensive material (Chalezkaya) that spleen and blood serum reduce susceptibility to a graft and inhibit the growth of a number of experimental tumors. But what is most important, spleen and blood from animals affected with carcinomas or sarcomas, whether transplanted, spontaneous, or produced by chemical agents, were found inactive in this respect. This observation led to a study of serum from normal and tumor-bearing animals, in the course of which it was found (Petrov and Chalezkaya) that while the former inhibits the growth of the Ehrlich carcinoma, the latter inhibits in but 8 to 10 per cent of cases.

The experiments just described have to do with factors that influence the established tumor. Others are under way concerning the possibility that antiblastomatogenic factors may exist. This study is only beginning, but we may communicate here some preliminary results.

It is an established fact that some chemical substances possess antigenic properties (Landsteiner and others), and it seemed worth while to ascertain whether or not some of the blastomatogenic compounds possess them. Two methods of attack were possible: (a) to administer the substances in pure form, or (b) combined with proteins. The latter has been adopted by Creech and Franks, who introduced dibenzanthracene into the protein molecule

[1] It would appear that some distinction should be made between mere loss of carcinogenic activity and the presence of an anti-carcinogenic factor, but the difficulties of international communication made it impossible to ascertain Professor Shabad's opinion on this. Ed.

and obtained in this way 1,2,5,6-dibenzanthranylcarbamidocasein, which possessed the specificity of anthracene.

We began with the former method. The study was carried out on 104 guinea pigs with *o*-aminoazotoluene, dimethylaminoazobenzene, 3,4-benzpyrene, and methylcholanthrene by the procedure of Landsteiner. Sensitization was achieved by means of a 1 per cent solution of the substance to be tested in pure olive oil, in doses of 0.1 cc. for 10 days in succession. Thirty days after the first injection a single injection of 0.1 cc. of a 1 per cent solution was made into the skin of the opposite side. All the experiments gave a negative result; no signs of a specific reaction, hyperergic inflammation or necrosis, were noted; there was no difference among the injection sites of various substances, or in the reaction of sensitized and control animals.

The combination of a carcinogen with proteins was then tried. *o*-Aminoazotoluene was tetrazotized and combined with the proteins of bovine and horse serum. The azoprotein thus obtained was used for sensitizing rabbits, whose serum was subsequently used to test the presence of specific precipitins. The latter were found to appear in the rabbit serum and to react specifically not only with the antigen used for sensitization, *e.g.,* the combination of *o*-aminoazotoluene with the proteins of bovine serum, but also with another antigen containing the same substance; that is, the combination of *o*-aminoazotoluene with the proteins of horse serum (Korosteleva). In this way it was shown that the combination of a carcinogenic compound with protein may possess antigenic properties that are specific for a definite blastomatogenic substance such as *o*-aminoazotoluene.

These data, of course, are only the first step in an entirely new field, and require further elaboration. Yet they suggest that within the organism there may exist antiblastomatogenic factors that counteract carcinogenic chemical compounds. Further progress along these lines may lead, on the one hand, to an attempt to influence exogenous, and eventually endogenous, carcinogenic agents. It is possible, on the other hand, that serological studies may contribute to the search for the latter by identifying them as antigens.

The Blastomatogenic Action of Some Substances of Direct Practical Significance

Study of the blastomatogenic compounds may throw light upon many aspects of tumor pathogenesis and at the same time of practical medicine. Thus it has been found that the latent period varies with the activity and dose of the compound administered, but on the whole it is fairly long, amounting, with agents of moderate activity like coal tar and 1,2,5,6-dibenzanthracene, to about one-fifth the life span. It will be noted that the latent period for occupational cancer in man is about the same, namely 12 to 15 years. This suggests the possibility of prophylaxis. If some product is suspected of being carcinogenic an experiment may show whether it is or not long before completion of the induction period in man.

Some time ago we investigated the slate tars of the U.S.S.R. and found (Larionov, Soboleva, and Shabad) that they differ in carcinogenic power. Thus the greatest number of tumors in mice was obtained with slate tar of Chuvashia (Middle Volga), a smaller number with Barsass tar (Western Siberia), while those of the Leningrad district were wholly ineffective.

Within recent years a number of studies have been carried out in this laboratory, which may be divided into 3 groups: (a) those dealing with lubricating oils, (b) with azo compounds, and (c) with hydrocarbons.

To the first group belong the studies of "sulfofresol" (Prokofieva) and "emulsol" (Verkhovskaya), both of which are used as refrigerating mixtures in the metal industry and contain mineral oils. They caused a pronounced dermatitis upon prolonged painting of the skin in mice. Sulfofresol induced papillomas in 25 to 45 per cent of the animals and cancer in 7 to 12 per cent at the site of administration, while emulsol produced papillomas in isolated cases only, and no cancer at all.

Certain azo compounds are of practical interest, since they may be used to synthesize food dyes. It should be emphasized in this connection that *o*-aminoazotoluene, and less so dimethylaminoazobenzene (Morozenskaya), may produce tumors of the liver and lungs upon application to the skin. This is to be borne in mind in considering the pathogenesis of aniline cancer of the urinary bladder in man.

Among the hydrocarbons of practical significance are some derivatives of 3,4,8,9-dibenzpyrene (Kleinenberg). While it is a very active carcinogen, its 5,10-quinone compound, which is an important industrial dye, is inactive either by itself or in the form of water-soluble compounds.

Here belongs also an experimental study on the properties of benzanthrene (Morozenskaya) and of tetramethyldiaminophenone, Michler's ketone (Prokofieva), which may serve as either initial or intermediate products in dye technology. These also were found to be ineffective with regard to tumor formation.

Experimental Cancer of Internal Organs

Though all blastomatogenic agents elicit multiple tumors at a distance from the site of administration, and primarily in the lungs, this does not enable us

to produce neoplasms of the internal organs at will, since to a certain degree these are a haphazard phenomenon.

More reliable is the adminstration of azo compounds. *o*-Aminoazotoluene, for example, elicits tumors first of all in the liver, thus providing a convenient method of investigating their earliest stages. This is exemplified in a recent study made in this laboratory by Elzina, who found that the respiration of liver cells did not decrease as the malignant transformation came on, and that glycolysis did not increase even at the stage of hepatoma formation. Transplantable hepatomas, on the other hand, behaved in all respects like any other grafted tumor.

A third approach to the experimental production of internal tumors is the introduction of carcinogens directly into the various organs. In this direction a number of studies have been carried out by Klenitzky, who produced cancer of the cervix in mice by inserting coal tar or 3,4,8,9-dibenzpyrene on cotton plugs. In this way the whole course of preliminary morphological changes was followed up and concepts of precancerous lesions of the cervix were modified. The experimental production of cancer of the cervix in mice is the more significant as spontaneous carcinoma at this site is rare in them, if, indeed, it occurs at all; but it was an elaborate procedure that failed in many cases. The simultaneous subcutaneous injection of folliculin favored the appearance of cervical cancer, whereas the administration of such an irritating agent as formol in vegetable oil did not cause either cancer or precancerous changes. In this way the significance of the combined action of different agents in the genesis of cancer of the cervix was demonstrated and the results of the experiments were brought closer to clinical pathology.

It was of interest to find that the introduction of dibenzpyrene plugs into the genital tract in castrated female mice produced no cancer at all. This undoubtedly points to the significance, as an endogenous blastomatogenic factor, of the sexual cycle. The latter is directly evidenced by the fact that papilloma and skin cancer in tar-painted mice appeared sooner and in greater number in females that had spent their lives isolated from males, in contradistinction to the smaller number of tumors recorded in females kept with males (Klenitzky).

The evidence just cited demonstrates the significance that can be attached to the female sex hormones and other estrogenic substances in the development of tumors. It seemed necessary, therefore, to investigate first of all the possibility that some carcinogenic substances may possess estrogenic activity. Prokofieva showed that, contrary to the widely adopted opinion based on the studies of English authors, not one of the numerous compounds tested, including 3,4-benzpyrene, had any such capacity.

Of great interest was the examination of synthetic estrogenic compounds for carcinogenicity. Lacassagne showed that the administration of large doses of folliculin produces malignant tumors in mice, but the question remains open whether it acts as a chronic stimulus to the genital apparatus by virtue of its powerful estrogenic action, or because it is a high molecular sterol. It seemed possible to approach this problem through a chemically more simple estrogen, polianol (Maksimov). This substance, which has a pronounced estrogenic action (Prokofieva), called forth considerable heterotopic proliferation of the genital epithelium in female mice (Kazanskaya), and peculiar adenomatous changes of the prostate with pronounced keratinization in male mice (Klucharev), but no tumors of any sort have so far been noted in spite of numerous and prolonged observations. Of special interest is the fact that the changes in the prostate receded as soon as the administration of polianol was discontinued (Klucharev). Thus the data point to the conclusion that a certain role in the blastomatogenic action of folliculin is played by its sterol structure but not by its physiological action.

As to the mode of action of estrogenic substances on the genital epithelium, it may be suggested that a direct stimulation takes place. This idea led to a study of the effect produced by folliculin on the healing of fissured nipples. According to Kazanskaya, painting the nipples of nursing mothers with folliculin promotes their healing.

In concluding this chapter of our review we may point out that the logic of research has led from investigation of the exogenous blastomatogenic agents to a consideration of certain substances, such as the female sex hormones, that may arise within the organism itself. We have demonstrated the significance in carcinogenesis of the combined action of exogenous and endogenous factors, in particular of the estrogenic substances mentioned above. The evidence thus obtained naturally brings us to a search for endogenous blastomatogenic substances that presumably may be produced within the organism itself and act as the cause of "spontaneous" tumors.

II. ENDOGENOUS BLASTOMATOGENIC SUBSTANCES

The development of the concept of blastomatogenic substances, their synthetic production, and the accumulation of a vast experimental material on their action, raise the question whether or not there may arise within the organism itself blastomatogenic substances similar in a certain degree to the exogenous agents known at present.

As early as 1925 Kennaway obtained tar-like car-

cinogenic substances through distillation of a number of organic products, including human skin, at a temperature of 800° to 920° C. In discussing the results he suggested that in the human organism similar substances may form although slowly and gradually. However, Kennaway himself insisted that his experiment should not be regarded as essentially different from those with coal tar: In both cases the responsible agent was a naturally or artificially produced exogenous compound.

A consideration of the structure of blastomatogenic substances in the light of modern knowledge of the structure of sex hormones, bile acids, and cholesterol justifies the assumption that within the organism there may occur complex polynuclear compounds resembling the blastomatogenic hydrocarbons (Cook).

An important contribution to this problem was Lacassagne's finding that in mice cancer may be incited by means of folliculin. Another, although indirect, piece of evidence to support this hypothesis was the production (Cook, and Fieser and his associates) of very active blastomatogenic substances, methylcholanthrene and cholanthrene, from bile acids. The possibility of the endogenous origin of blastomatogenic substances was indicated also by some additional but still less direct evidence. Thus Burrows and Mayneord injected mice subcutaneously with a lard solution of cholesterol that had been irradiated with a large dose of x-rays. Sarcoma arose at the site of injection in 2 animals. Of great interest is Bittner's demonstration that the agent causing mammary tumors in certain strains of mice is transmitted through the milk.

The indirect evidence just cited was obviously insufficient to solve the problem of the endogenous origin of blastomatogenic substances. It could be solved only by direct experimental attack; by endeavoring to isolate from cancer patients chemical substances that would produce tumors in animals. At the present time we have information on futile attempts in this direction by several authors (Bricker, Bürger and Uiker, Rondoni, Sobotka and Block, etc.). Such failures are quite comprehensible, since direct experiment involves a number of enormous difficulties. As a matter of fact, prior to our studies, which offered the first direct evidence of endogenous blastomatogenic substances in man, it was uncertain how to search for them and where, and what is to be regarded as a blastomatogenic agent within the human body. Moreover, one had to take account of the fact that blastomatogenic substances might exist within the human body only at certain stages in malignant neoplastic disease, and hence might be absent when the attempt is made to discover them. This suggestion seems especially pertinent in view of the well known fact that tumors in animals may appear a long time after administration of an exogenous agent has been discontinued. Despite these difficulties we are able to offer considerable experimental evidence for the occurrence of blastomatogenic substances within the human body.

The first experiments, carried out by the author in 1935, consisted in painting mice with a 1½ month benzene "infusion" (in the cold) of minced liver or tumor from persons dead of cancer. The experiments were carried out on 162 mice, but could not be completed because nearly all the animals died within 3 to 5 months from the toxicity of the preparation. None of the animals developed a tumor, but it should be borne in mind that none of them lived more than 7½ months from the beginning of the experiment.

This failure forced us to modify the experiment by concentrating the extracts and administering them subcutaneously. In January, 1937, malignant tumors were recorded at the injection site in 3 out of 8 mice treated with benzene extracts of the liver of a woman dead of gastric cancer. Since then a great number of experiments have been carried out in this laboratory, most of which have already been described (Shabad, Neufach, Kleinenberg). It was found that liver extracts from cancer patients actually contain blastomatogenic substances, and our pioneer results have been confirmed by others (Hieger, Des Ligneris, Steiner, and so on). Below we discuss briefly our principal results.

Liver Extracts

Benzene, a reliable solvent for a number of synthetic carcinogenic compounds, was used to extract blastomatogenic substances from the human body. Relying upon observations on remote tumors, we considered the possibility that blastomatogenic substances may circulate throughout the organism, and it seemed worth while to try to extract them from some organ that was not involved by the tumor. It was natural to attack first of all the liver, which is closely connected with the transformation of sterols and is the site of formation of bile acids. Furthermore, the liver seemed advantageous because of its large size.

It was accordingly removed from 41 persons dead of malignant growths at various sites. In most cases, 14, the tumor affected the stomach or, in 6, the lungs. All other localizations and varieties of tumor were represented by single cases. In 32 of the 41 cases the extracts were prepared from livers in which no metastases could be detected macroscopically. In the remaining 9, the livers contained large metastases, and the extracts were prepared not so much from liver itself as from the invading malignant tissue.

Each extract was used for a single experiment as a rule. The number of mice employed varied ac-

cording to the amount administered (about 1 gm.). In some instances undissolved extract was employed whereas in others the extract was diluted 2 to 3 times with olive oil because of its toxicity. Two hundred and seventy-three mice were used, of which 179 belonged to strain RV.

Livers from 26 persons dead of various diseases, and not affected with cancer, served as controls. Their ages varied from 20 to 80 years, 19 of them being above 40, in accordance with the age of the cancer patients. Twelve had died of pneumonia and 10 of cardiovascular disorders. The diagnosis, as in all other cases examined, was confirmed by autopsy and microscopic examination.

Bile Extracts

In order to restrict as much as possible the large number of ingredients of liver tissue, a series of experiments was carried out (Neufach and Shabad) with bile extracts. The bile was procured from the gall bladders of persons dead of gastric cancer (2 cases), lung cancer (2 cases), and sarcoma (2 cases). The bile was evaporated to dryness on a water bath and then extracted with benzene; the benzene was distilled off and the remainder dissolved in olive oil and lard and injected subcutaneously into 35 mice.

All the mice of our strain RV that served as controls lived out their natural span, and at death were subjected to careful macroscopic and microscopic examination. Of 634 that died throughout the observation period 389 were above 8 months old, and in 40 of them several kinds of tumors were detected; *i.e.*, 6.3 per cent of the total number, or 10.2 per cent of those surviving more than 8 months. Malignant tumors occurred only 9 times, thus in 1.4 or 2.3 per cent. First place as regards frequency was occupied by primary adenoma of the lung, detected in 33 cases (5.2 or 8.48 per cent); in 4 of these the growths were malignant. Other neoplasms, cancer of the skin, hepatoma, etc., occurred only occasionally. Adenocarcinoma of the mamma was found in 4 animals, *i.e.*, in 1.2 or 2.03 per cent of the female mice. In one female there was a large adenoma.

Comparison of these results with those obtained in mice injected with liver extracts from persons dead of cancer showed a considerable increase in the number of tumors for the latter group. Among 179 mice of this series, or among the 108 that lived more than 8 months, some variety of tumor was found in 62; in 24 the growth was malignant. Thus the percentage of mice with tumors was 34.6 or 57.4 per cent as compared with 6.3 or 10.2 per cent in the controls, and the percentage with malignant tumors was 13.4 or 22.2 against 1.4 or 2.3 per cent. That is to say, the total number of tumors was increased 5 times, and that of malignant neoplasms as much as 10 times. It will be of interest to note that the administration of bile extracts from persons dead of malignant tumors gave approximately the same results as the liver extracts of these patients. Thus with bile extracts tumors were recorded in 37.1 per cent of all animals, or in 50 per cent of mice surviving more than 8 months, while the number of mice with malignant tumors was 17.1 per cent of the total number, or 23 per cent of those that survived for more than 8 months from birth.

Analysis of the material according to the variety and localization of the tumors showed that in every category the number of tumors was appreciably greater in mice treated with liver and bile extracts from cancer patients. This was true of cancer of the skin and the mammary glands, as well as of lung tumors. Moreover, among the mice injected with the extracts there occurred tumors such as were not found in the controls; *e.g.*, squamous cell cancer of the jaw, or cancer of the kidney.

Finally, in 6 mice (2 males and 4 females), that is, in 3.3 or 5.5 per cent, we noted malignant tumors at the site of injection of liver extracts from cancer patients. In 3 these were sarcoma, in 1 carcinosarcoma, and in 2 mammary adenocarcinoma. As a rule these growths were connected with the oleoma. As to their morphology, they resembled in every respect malignant tumors elicited at the site of injection of synthetic carcinogenic compounds. Two of the sarcomas were transplanted with success and carried to the 31st and 45th generation respectively. The adenocarcinoma failed to grow after transplantation.

In mice injected with liver extracts from persons that did not die of cancer the tumors were of significantly less frequent occurrence, although more numerous than is the rule with untreated mice of our strain. The total number with tumors was 2 to 2½ times greater than in uninjected mice, and about that many times less than after the injection of extracts from cancer patients. Malignant tumors were detected after the administration of "noncancer" extracts only twice as frequently as in the untreated group, and about 4 times less frequently than with "cancer" extracts. It should be emphasized that the overwhelming majority of the tumors occurring after the injection of noncancer extracts were lung tumors, and that other varieties and localizations were represented by single cases. No tumors occurred at the injection site.

Thus it will be seen that the tumor frequency was significantly different in mice treated with cancer and noncancer extracts. The number produced by liver and bile extracts was so much above that spontaneously arising in our strain that there can be hardly

any doubt as to the blastomatogenic effect of the extracts.

Lung Extracts

Our attention was primarily attracted to the lungs as they appear to play a certain role in the metabolism of lipoids, which may act as solvents of blastomatogenic substances. Moreover, as has been indicated above, primary tumors of the lung are fairly frequent after the injection of exogenous blastomatogenic substances, which is to be accounted for by distribution of these substances throughout the organism.

The lungs of 19 persons dead of malignant tumors at various sites (8 of gastric carcinoma, 1 of sarcoma) were extracted. In only 3 did the lungs contain tumor tissue (2 cases of primary cancer of the lung and 1 with metastases from a sarcoma). As controls we used the lungs of 20 patients, 12 of whom died of pneumonia. The experiments were carried out on 212 mice.

In mice injected with extracts from persons dead of malignant neoplasms there appeared a great number of tumors. Thus among mice surviving more than 8 months from birth various kinds of tumors were detected in 54.3 per cent, the percentage of malignant ones amounting to 13.8 per cent. It should be emphasized that these data coincide with those obtained upon the injection of bile and liver extracts (50.0 or 57.4 per cent). Extracts from persons dead of diseases other than neoplasia produced a much smaller number of tumors; 13.6 or 23.1 per cent against 37.3 or 54.3 per cent, although this is notably greater than the spontaneous tumor incidence (6.3 or 10.2 per cent) in mice of the RV strain.

Particular attention is called to the fact that in one case we succeeded in inducing sarcoma at the site of injection of an extract of the lungs of an 89 year old woman with cancer of the gall bladder that had not metastasized to the lungs.

In this same series another mouse, which died 16 months after the beginning of the experiment, had a keratinizing squamous cell cancer of the mouth.[2] The total number of cancers of the mouth obtained with lung extracts from persons dead of malignant tumors amounted to 3, whereas with noncancer extracts and in untreated mice of the RV strain no such tumors ever occurred.

The greatest number of tumors was always found in the lungs. Except for one carcinoma, these were adenomas. Cancer extracts produced lung tumors in 31.3 or 47.8 per cent of injected mice, and noncancer extracts in 13.6 or 23.2 per cent. Spontaneous lung tumors in the RV strain occurred in 5.2 or 8.48 per cent of the mice.

The results obtained testify to the possibility of detecting blastomatogenic substances in the liver and lungs of man, that can be extracted with benzene. This confirms all our preceding statements, and contributes further to an elucidation of the nature of the blastomatogenic agents in the human organism, since it excludes a number of products characteristic of hepatic tissue, such as a great number of pigments, bile acids, etc. Finally, lung extracts displayed a less toxic and irritating effect than those of liver and bile, though they produced as many tumors.

Nonsaponifiable Fraction

The crude benzene extracts of the preceding experiments contained a mixture of most diverse substances, mostly of a lipid nature. The fractionation of lipid organ extracts, with a simultaneous assay of the blastomatogenic activity of every fraction, was greatly impeded by the fact that results can be obtained only by the expenditure of a large number of animals and considerable time. Hence we had to confine our study to one fraction only. For this purpose the nonsaponifiable fraction of liver extracts from cancer patients was used.

In an experiment carried out on 50 mice we used the livers from 9 patients (2 men and 7 women) dead at from 37 to 65 years of cancer at various sites (stomach in 6 cases, liver [primary] in 1, uterus in 1, and ovary in 1). In 4 of these 9 cases a large number of metastases were seen in the liver. Thus, in a total of 5 extraction was done not so much on hepatic tissue as on invading tumor.

The administration of the nonsaponifiable fraction elicited various kinds of tumors in 31.2 per cent of the mice that lived to be more than 8 months old. Particular attention is directed to the fact that in 1 case we succeeded in obtaining a sarcoma at the injection site, while the total number of tumors was 2 to 2½ times as great as that occurring spontaneously in our mice.

DISCUSSION

The total number of animals treated with extracts amounted to about 800. The material was procured from over 100 human bodies (liver in 76 cases, lungs in 39, and bile in 6).

It is concluded that the injection of benzene extracts of the liver, bile, and lungs from persons dead of malignant neoplasms produces diverse tumors in mice, either benign or malignant, and either at the injection site or, most often, distant from it.

It is to be pointed out that our extracts were prepared from the livers, bile, and lungs of persons

[2] Attention is called to the fact that the liver extract of the same patient likewise possessed an appreciable blastomatogenic capacity.

affected with various forms of malignant tumors at different sites. The results obtained should therefore be related not to some special property of cancer of the stomach, bronchus, gall bladder, etc., but to some property common to all malignant tumors. At the same time, in contrast to Steiner and some others, we did not combine extracts from different patients; hence we may state with certainty that the blastomatogenic effect was produced by diverse malignant tumors; by cancer of the stomach, lung, larynx, intestine, pancreas, gall bladder, liver, and by lymphosarcoma, of both men and women.

The results obtained with the nonsaponifiable fraction from cancer patients suggest that the blastomatogenic substances detected in man belong among the nonsaponifiable compounds. Attention is therefore directed to the possible transformations of sterols and their role in the genesis of cancer, particularly under the influence of such factors as radiant energy. This is particularly important in connection with the tumors caused in both man and the lower animals by x-rays, radium, ultraviolet rays, etc. In this connection it may be mentioned that Neufach, of this laboratory, has demonstrated the transformation of cholesterol by ultraviolet irradiation into a peculiar product possessing certain new properties; *i.e.,* change of color, melting point, absorption spectrum, fluorescence, and the capacity to affect a photographic plate. Khaletzkaya, on the other hand, showed in this laboratory that in mice painted with blastomatogenic substances there occurs during the papilloma, or initiatory, period a rise in the blood cholesterol.

The production of tumors with bile and certain organs from cancer patients was so unexpected that doubt has been aroused concerning the reliability of the result.

First of all, one might regard the growths as spontaneous, and hence not connected with the administration of the extracts. This doubt is refuted by at least two considerations: (a) In most of our experiments mice of a known strain were used, and a sufficient number of control animals (above 600) enabled us to determine the incidence of spontaneous neoplasms. (b) Different percentages of tumors were obtained with extracts derived from cancer patients and from persons dead of other diseases. It should be emphasized that tumors were recorded at the site of injection, and that these were produced only by extracts from cancer patients.

A second ground for argument is the suggestion that the blastomatogenic substance was formed during preparation of the extracts. The objection may be refuted for the following reasons: Benzene cannot be responsible for the blastomatogenic action, since it has long been known that the low-boiling tar fractions, to which benzene belongs, are not carcinogenic. Furthermore, control experiments showed that benzene does not produce either local or remote tumors, lung tumors in particular. The extracts were not heated above 80° to 140° C. and did not reach those temperatures (800° to 900° C.) at which Kennaway and others have obtained carcinogenic products from various organic materials. But the most conclusive evidence that the active principle in our extracts actually was isolated from the human body is the fact that the tumors produced by material from cancer patients were more numerous that those incited by extracts from patients dead of other diseases. The fact that similar results were obtained with liver, lung, and bile extracts seems to support the idea that neither the extraction nor the conditions under which the material was procured will explain the blastomatogenic action of our extracts.

Finally, a third possible objection is that the tumors in our experiments were not produced by specific blastomatogenic substances, but by nonspecific irritation associated with the chronic inflammation at the injection site. Against this are the numerous observations on chemically pure carcinogens, which show that there is no correlation between tumor formation and irritation. Secondly, it deserves to be emphasized that bile extracts, which are more irritating than liver extracts, produced no tumors at the injection site, whereas lung extracts, which are less irritating, elicited sarcoma. In the third place, liver extracts from persons free of cancer were no less irritating than those from cancer patients, yet they produced a significantly smaller number of tumors. Finally, and most important, nonspecific irritation through local chronic inflammation cannot account for the appearance of remote tumors. Since these have appeared in connection with tar and synthetic carcinogenic agents also, they can be explained only by a general effect of the agent upon the organism. And this points to the conclusion that the liver, bile, and lung extracts actually contained blastomatogenic substances.

Our first communication stimulated others to repeat our experiments. Butenandt did not succeed in obtaining tumors in mice, apparently because his nonsaponifiable fraction was administered only once; that is to say, probably in too small a dose. Gummel also failed to reproduce our results.

The first confirmation was reported by Hieger, from Kennaway's laboratory. Among 367 mice treated with various kinds of liver extracts from persons dead of malignant tumors or other affections, he obtained sarcoma at the site of injection in 13. Most of these (11 of 13) were produced with extracts from persons with malignant tumors; only 2 resulted from the in-

jection of extracts from Bantu natives dead of "uncertain causes, but not cancer." It will be remembered that among the Bantus of South Africa, from whom Hieger obtained his material, primary cancer of the liver is of comparatively frequent occurrence.

Des Ligneris carried out his study in cooperation with Hieger, by painting the skin of mice with liver extracts from persons dead either of cancer or of other diseases. Among 237 mice he obtained 25 tumors in the painted area, and of these 7 proved to be malignant.

Steiner administered subcutaneously the nonsaponifiable fraction of liver extracts from persons dead of cancer, and among 37 mice that survived for more than 6 months from the beginning of the experiment obtained a sarcoma at the injection site in 13. He denied the blastomatogenic activity of similarly prepared liver extracts from patients free of cancer, but subsequently encountered the phenomenon as did we, although the number of tumors was smaller than with extracts from cancer patients. Similar extracts of the neoplasm itself did not produce any tumors in Steiner's experiments, but Menke obtained sarcomas in 2 mice at the injection site of an extract of cancer from the human breast. Yet Menke did not succeed in producing tumors with the nonsaponifiable fraction of cancer extract.

Kinosita reported that Tanaka elicited sarcoma in mice at the injection site of the nonsaponifiable fraction of liver extract from a man dead of cancer of the stomach. Similar results were reported also by Sannié, Truhaut, and Guérin, in France. Finally, Steele, Koch, and Steiner have described the production of tumors with extracts of urine from patients with and without cancer.

Thus the possibility of detecting carcinogenic agents in the human body, first reported by the writer, has been confirmed. In numerous experiments, carried out in this laboratory as well as in other countries and on different strains of mice, tumors have been produced with extracts of bile, liver, lungs, and urine from persons dead of malignant growths. It is significant that owing to modifications in the preparation, and particularly in the application, of the nonsaponifiable fraction in both this and other laboratories, it is certain that the origin of the blastomatogenic substances is not referable to such chemical procedures as the use of benzene, etc.

Of special interest is the blastomatogenic effect of extracts from persons dead not of cancer, but of other diseases. According to our experience, which has been confirmed by Steiner as well as by Sannié, Truhaut, and Guérin, this is possible, and it may be accounted for in two ways. First, all livers may accumulate small amounts of blastomatogenic substances, which manifest no activity probably because of insufficient concentration. Secondly, among the controls there may have been persons who would have developed cancer had they not died of some other disease. This second alternative is supported in part by the data of Hieger and Des Ligneris on the livers of Bantus, though no decisive conclusion can yet be drawn. In any case, our data and those of Hieger, Des Ligneris, and Steiner leave no doubt that the blastomatogenic activity of extracts from persons dead of cancer is more pronounced than that of similar extracts from those free of the disease. This means either that extracts of the liver and lungs from persons dead of malignant tumors contain more blastomatogenic substances than extracts from those without cancer, or that their action is more effective.

In most of our experiments the extracts were prepared from livers and lungs that were free of metastases; hence it may be taken for granted that blastomatogenic substances may be found in the human body at a distance from the tumor. Some experiments, however, were done with livers containing so many metastases that we actually dealt with a tumor extract rather than an extract of the liver itself. These were much less active than extracts from livers without metastases, eliciting 27 per cent of tumors against the 62 per cent obtained in mice injected with extracts of livers that were free of metastases. It will therefore be seen that a tumor may contain at least no more blastomatogenic substances than the liver. This conclusion is supported by Steiner's data and by the unpublished data of Khaletzkaya, of this laboratory, and of Vadova (laboratory of Professor N. N. Petrov), which show that benzene extracts of the Ehrlich mouse carcinoma and the Brown-Pierce rabbit carcinoma produce no tumors in mice or rats at the site of administration.

Although this important question of the localization of blastomatogenic substances in tumors still remains open, it is our definite opinion that they neither produce nor accumulate blastomatogenic substances of the type with which we are now concerned.

In considering the results of our experiments and those confirming them, the fact deserves mention that the number of tumors elicited was not great in all the experiments with extracts from persons dead of malignant tumors. It will be recalled that in our own experiments we did not as a rule combine extracts from different sources, and so had a better chance to compare the presence and the amount of blastomatogenic substances in various subjects.

The results appear to suggest that blastomatogenic substances can be isolated from the organs of some persons only and that not all extracts, even from cancer patients, have considerable blastomatogenic

activity. With regard to this fundamental problem it should be borne in mind, however, that the determination of blastomatogenic effect involves great difficulties. Indeed, even preparation of the extracts is not yet standardized, while an actual estimation of their effect requires 2 years of observation on mice whose age and intercurrent diseases may affect the outcome of the experiment.

One of the most essential problems to be solved in the future is whether the blastomatogenic substances in the human body are of exogenous or endogenous origin. This cannot be settled until we have more information on their chemical nature; and, what is most important, on the conditions governing their presence in the organism. Even then it will not be easy to decide. We may recall as an example that cholesterol originates within the organism, but it can also be ingested with the food. Nevertheless, it may be suggested provisionally that the blastomatogenic substances in question are most probably of endogenous origin, in the strict sense of the term.

The last question to be discussed is that of the chemical nature of these endogenous blastomatogenic substances, and in particular of their specificity. Though their nature is still wholly obscure, it may be said at least that they are very stable; extractable by benzene, petroleum ether, and other organic solvents; and, what is most important, that they belong to the nonsaponifiable fraction of the lipids in common with cholesterol, the bile acids, and allied substances.

In this connection it might have been thought that the blastomatogenic effect noted in our experiments was exerted not by some new and still unknown product, but by certain constant constituents of the liver such, for example, as the bile acids. The suggestion might be strengthened by the fact that Ghiron, and later Cook and the Kennaways, showed that the administration to mice of desoxycholic acid may eventually elicit sarcoma. Yet it appears that there is enough evidence at present to contradict the idea that bile acids are the active principle of our blastomatogenic extracts. The amount of chemically pure bile acids which, according to Cook and Kennaway, produces malignant tumors in mice is as much as 70 mgm., or at least not less than 28 mgm. This greatly exceeds the quantity in the amounts of liver extract injected. Again, not all livers by any means contain a similar quantity of the active principle, as is conclusively indicated by our data and as has been mentioned by Hieger also. It will be of interest to note that, according to Steiner, the total amount of nonsaponifiable lipids is about the same in livers from persons with or without cancer. The appreciable difference in the number of tumors elicited by liver extracts from cancer patients and those dead of other affections testifies against a blastomatogenic role for the bile acids. If it be assumed that the bile acids of cancer patients are distinguished by some unusual properties, in particular by a specific blastomatogenic activity, we come back once more to a denial of the blastomatogenic role of bile acids from the normal liver and the assumption of special blastomatogenic substances in the livers of cancer patients. Finally, the blastomatogenic activity of the lungs, whose tissue components differ essentially from those of the liver, naturally contradicts the blastomatogenic role of bile acids.

It may thus be assumed that there exist within the human organism blastomatogenic substances that behave to a certain degree as specific chemical originators of malignant neoplasms. One may readily imagine that it is precisely with the appearance of these that the "prehistory" of cancer begins. Hence the problem of neoplasia becomes a double problem, in which not only the condition of the cell "stimulated" by the carcinogenic agent has to be explained, but the origin and character of the "stimulus" itself accounted for.

The question naturally arises whether there exist factors that counteract both the production and activity of endogenous blastomatogenic substances and thereby impair their capacity to produce tumors. The study of exogenous blastomatogenic agents may give some information here, for it is known that a change in structure may deprive them of their activity. On the other hand, certain reactions of the organism that tend to detoxicate these compounds should be investigated.

SUMMARY

The data so far supplied by the study of experimental carcinogenesis suggest certain general concepts on the origin of tumors. When the two principal hypotheses, those of Virchow and of Cohnheim, are examined from the modern point of view it will be seen that both have been about equally confirmed and discredited. Virchow's conception of the importance of irritation has been justified in so far as it led gradually to the discovery of the chemical carcinogens, but the modern concept of blastomatogenic "stimulation" is substantially different from the old idea of nonspecific irritation. As to Cohnheim's hypothesis, any carcinogen may elicit a tumor at any site in any animal, which makes the presence of embryonic remnants unnecessary. Yet if the blastomatogenic substance is endogenous it is only a cell product after all, and in this respect the modern point of view approaches more or less that of Cohnheim, who especially accentuated the significance of the endogenous factor in tumor genesis.

The origin of endogenous blastomatogenic substances and the mode of their action remain the fundamental problem in cancer research. Although it is still obscure, the recent rapid accumulation of important information on the etiology and pathogenesis of neoplasms stimulates us to still more tenacious and thorough investigation, and encourages a belief in final success.

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NOTE: We have conscientiously endeavored to verify all references, in accordance with our custom, but because of war conditions many of the publications here cited are unavailable.—Ed.

Methylcholanthrene Papillomas and the Virus Problem

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Papillomas of proved virus origin are no rarity in the animal kingdom (1, 5, 6, 7), but none has been so minutely investigated as the Shope papilloma of the rabbit (8). Though this tumor yields virus in generous amounts when it is grown in western cottontail rabbits, the etiological agent can no longer be obtained (4) from the carcinomas into which the warts in these hosts are occasionally transformed (4, 9).

Does an analogous situation obtain with induced papillomas of the mouse and the carcinomas that arise from them? The answer to this question will be found in the following paragraphs.

MATERIALS AND METHODS

To obtain the necessary papillomas, young adult RIII male and female mice of a brother-to-sister bred strain were painted thrice weekly with a 0.6 per cent solution of methylcholanthrene in benzol.

It is not easy to decide on the exact day upon which a papilloma first appears, but those employed were judged to be about 7, 11, 18, 42, 51, 82, and 114 days old.

Only such warts were used as showed no sign of thickening at the base, an indication of the malignant transformation that is conceded to be trustworthy and that had to be relied upon in any case, since there was not sufficient material available for microscopic examination.

As papillomas were required they were excised, after they and the surrounding skin had been gently cleansed with a cotton pledget just barely moistened with benzol to remove any adherent carcinogen, then with cotton similarly wetted with alcohol to get rid of the benzol, and immediately mopped dry with sterile cotton. It is not thought that either of the cleansing fluids thus used could have destroyed any virus present.

It was necessary to kill three or four mice each time in order to get sufficient material. After the warts had been removed the 0.3 to 0.6 gm. of tissue so obtained was thoroughly ground with sand in physiological saline solution and the resulting extracts were centrifuged for 15 minutes at about 2,000 r. p. m. They were then either watery-clear or faintly opalescent, and sometimes lightly blood-tinged. Had any of them elicited tumors, adequate filtration of subsequent extracts would of course have been required.

Thus prepared, the extracts were inoculated in the usual way, by rubbing them with a glass rod into shaven skin that had been lightly scarified with a needle. For this purpose young adult male and female mice of the strain that furnished the papillomas were employed, with resort in most cases to procedures that it was hoped might enhance the susceptibility of the organism or the efficacy of the extracts. The details appear below.

Experiment 1.—A 5 per cent papilloma extract was rubbed into the scarified but otherwise normal skin of 6 male and 6 female mice.

Experiment 2.—Similar, except that a 10 per cent extract was used.

Experiment 3.—Here the skin was made hyperplastic by painting it with equal parts of turpentine and acetone, the method so successfully employed by Friedewald (2), who increased the effective titer of the Shope papilloma virus from 10 to 100 times in this way. Twelve male and 12 female mice were thus treated, but the skin of this RIII strain seems to be less delicate than that of the rabbits described by Friedewald, for 9 applications had to be made, at intervals of 2 days, before the skin reached the thickened and inflamed condition that he achieved with 4 or 5 paintings at similar intervals. Into this hyperplastic skin a 10 per cent papilloma extract was rubbed after the customary scarification.

Experiment 4.—It has been said that some viruses gain efficacy as a result of glycerol storage in the cold. Such an occurrence must be rare if, indeed, it takes place at all, for it is clearly stated of most viruses that they lose strength with the passage of time. Nevertheless, and as a forlorn hope, some papillomas were stored in the refrigerator in equal parts of glycerol and physiological saline solution for 48 days. At the end of this period the glycerol was washed away in 3 changes of saline solution, and the usual 10 per cent extract was inoculated into 12 male and 12 female mice whose skins had been previously irritated with the turpentine-acetone mixture.

Experiment 5.—An effort was made to diminish the resistance of the animals by exposing their entire bodies to as large a dose of x-rays as a mouse can safely stand. Six males and 6 females received 300 r (184 kv. peak, 2 mm. Cu + 1 mm. Al filter), and a similar dose 6 days later. Twenty-four hours after the second raying the white cell count in the females had fallen to 4,500, in the males to 2,750, and on the following day all were inoculated in the customary manner with a 10 per cent papilloma extract.

Experiment 6.—The inoculated area was covered immediately with paraffined gauze, which was held in place by adhesive plaster until healing had occurred, about 5 days later. According to Friedewald (3), necrosis and scabbing are almost entirely obviated by this procedure, many susceptible cells are provided for the virus far earlier than they otherwise would be, and the inoculum is conserved instead of being largely lost amidst necrotic tissue and scab. The experiment comprised 12 male and 12 female mice inoculated with a 10 per cent extract of papilloma.

Experiment 7.—Similar to Experiment 6 except that the skin was made hyperplastic before the inoculation, for Friedewald (3) estimates that the combination of inflamed skin and a paraffined gauze pad increased the efficacy of the Shope papilloma virus from 100 to 10,000 times. Eleven male and 12 female mice were employed.

DISCUSSION

Though the incubation period of the Shope rabbit papilloma falls well within 2 weeks, the mice of these experiments were kept under observation for many months. Yet among 131 animals not the slightest indication of a papilloma has appeared. The scarifications have healed smoothly in a week or so and the skin has regained its normal appearance in every instance.

Nevertheless it would be unsafe to conclude that methylcholanthrene papillomas do not contain a virus. All that can be said is that none was disclosed under the conditions governing these experiments.

It may be of significance that viruses are recoverable from certain forms of leukemia in fowls and from many of their tumors, but not from mouse tumors or mouse leukemia. It is not beyond the bounds of possibility that the mouse holds on more tenaciously to some of its viruses or that some of them are more labile than certain avian viruses.

SUMMARY

Of 131 mice inoculated with extracts of methylcholanthrene papillomas, not a single one developed a tumor.

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Effect of X-Rays on the Transmissibility of Fowl Sarcoma in Its Nonfilterable Phase*

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It is a recognized fact that the filterable fowl tumors are remarkably resistant to roentgen radiation (13, 23, 24, 28, 30, 31). This resistance is due to the extreme refractoriness of the causative agent to x-rays. There is, however, no information as yet concerning the radiosensitivity of virus-induced fowl tumors during the period in which they have temporarily lost their filterability. The following experiments were designed to determine the effect of x-ray doses lethal to the cells upon the sarcoma during this so-called nonfilterable phase.

MATERIAL AND TECHNIC

Tumor material.—We used a strain of Rous sarcoma, kindly placed at our disposal by Dr. A. Fischer, of Copenhagen, that has been kept *in vitro* since 1923 (9). Inoculation into young chickens is successful in almost 100 per cent. The tumor generally leads to the death of the fowl in 4 to 8 weeks, and metastases are of regular occurrence.

Extraction and filtration.—The filtrates used in the present experiments were prepared in the following way: Three to five grams of tumor, freed of necrotic areas, was finely minced, thoroughly ground with sterile sand, and extracted with 20 cc. of 5 per cent NaCl for 1 hour. Eighty cubic centimeters of distilled water were then added, the suspension was centrifuged at 3,000 r.p.m. for 15 minutes, and the supernatant fluid drawn off and passed through a filter. Two types of filters were used: (a) paper-pulp-sand filter, and (b) Berkefeld "N" candle. The filtration was performed at a pressure of 20 mm. of mercury.

Irradiation.—Irradiations were carried out with a demountable x-ray tube working at 35 kv. on a current of 15 ma. The tube had a copper anticathode, and a window of aluminum foil 30μ in thickness. Absorption analysis showed that the rays penetrating through the window foil and the 0.03 mm. mica coverslip were mainly the characteristic x-rays of copper. Radiation was administered at a target-object distance of 3.7 cm.; the x-ray intensity at the distance of the irradiated object was about 80,000 r/min. for a tube current of 15 ma.

The tumor fragments (30 to 50 mgm.) were placed on a depression slide, covered with a mica coverslip, and sealed in with paraffin. The material was irradiated with 50,000 r, this being 10 times the dose necessary to prevent further multiplication of the cells (7).

Inoculation.—White leghorns, 2 to 6 months old, were used. The irradiated fragments were deposited in a pocket made in the muscle of the breast or of the leg. The filtrates were injected intramuscularly, 1 cc. being used for each injection.

Design of experiments.—Ten series of experiments with 10 different tumor samples were carried out. Hard, yellowish, slowly growing tumors were generally selected, and Berkefeld and paper-pulp-sand filtrates were made from each. In addition, small pieces of the growth were irradiated. The irradiated material and one or both filtrates were inoculated into the same chicken. The activity of the original tumor material was also tested in every case.

RESULTS

The results (Table I) may be summarized as follows:

(a) Berkefeld filtrate gave the most irregular results, only 3 out of 10 tumors having yielded active filtrates. Paper-pulp-sand filtrates were more effective, positive results having been obtained in 5 out of 10 experiments.

(b) Grafts of irradiated tumor gave positive results more frequently than either of the filtrates. In Experiments 1, 5, and 10 both Berkefeld and paper-pulp-sand filtrates were inactive, whereas irradiated tumor gave rise to growths.

(c) In some cases the irradiated tumor failed on inoculation. Thus in Experiments 3 and 6 not only both filtrates but the irradiated material, too, was found to be innocuous.

* Because of the difficulties of international communication the authors have not read proof of this article.

** Working under the Cancer Laboratories Fellowship.

TABLE I

Tumor no.	Chicken	Length of life, days	Results of inoculation with: X-rayed tumor	Paper-pulp-sand filtrate	Berkfeld filtrate
1	A	65	+	—	—
	B	66	++		—
	C	38	+	—	
2	A	24	—	++	
	B	23	+		++
	C	60	++	—	
	D	22	+	—	+
3	A	53	—	—	—
	B	53	—	—	—
	C	26	—	—	—
	D	45	—	—	—
4	A	11	++		+
	B	27	+	++	
	C	23	+	+	
	D	20	+		—
	E	26	++	—	
5	A	44	+	—	—
	B	16	+	—	—
	C	16	—	—	—
	D	39	+	—	—
6†	A	61	—	—	—
	B	70	—	—	—
	C	55	—	—	—
	D	36	—	—	—
7	A	28	+	—	—
	B	23	+	—	—
	C	186	+	—	—
	D	27	++	+	—
8‡	A	42	—		—
	B	25	+	++	++
	C	54	—	++	
	D	48	+	+	+
	E	14	+		+
9	A	23	+	+	—
	B	31	++	+	—
	C	104	+	+	—
	D	18	+	—	—
	E	40	++	+	—
10	A	43	—		—
	B	78	—	—	
	C	78	—	—	
	D	97	—		—
	E	97	—	—	—
	F	32	++		—

\+ = slowly growing tumor.
++ = rapidly growing tumor.
— = no tumor.
† Tumor No. 6 developed in a resistant fowl that had failed to respond to 2 previous inoculations of tumor.
‡ Tumor No. 8 was derived from tumor No. 1, which yielded inactive Berkefeld and paper-pulp-sand filtrates.

DISCUSSION

The filterability of chicken tumors is subject to considerable fluctuation (14, 32), varying not only with different strains, but also within an individual strain. Even those strains that are known to be easily filterable (*e.g.*, Rous sarcoma I) pass through non-filterable phases, in which their filterability is diminished or temporarily lost.

There is a considerable bulk of evidence to indicate that the distinction between filterable and nonfilterable fowl tumors is to a great extent an artificial one. By the use of appropriate filters and adequate methods of

pretreatment it is possible to improve the effectiveness of filtration considerably (2, 10, 15, 18, 35, 36). But the fact still remains that even when tested with better methods of extraction and filtration tumors often yield filtrates that are unaccountably inactive.

In order to study more closely the problem of the cell-free transmissibility of fowl tumors, Cramer and Foulds (6) undertook experiments to test for the presence of the causative agent by a method other than filtration. These authors investigated the transmissibility of fowl tumors that had previously been subjected to repeated freezing and thawing, and were able to show that the failure of cell-free transmission in the nonfilterable phase is not caused by the failure of the filtration method alone. The frozen tissue displayed variations in infectiveness similar to those of filtrates prepared from the same tumors, and sometimes it was completely inactive.

The present work represents an attempt to gain some further information on the cell-free transfer of a virus-induced fowl sarcoma in its nonfilterable phase, by investigating the transmissibility of tumor material previously irradiated with x-ray doses lethal for the cells. The results were compared with those obtained by inoculation of filtrates from the same tumors.

The application of irradiation as a method for separating the causative agent from living cells (12, 13, 16, 28, 31, 33) is based on the observation that a wide gap exists between the doses necessary for inactivation of the causative agent and those that prevent a further multiplication of cells. It has been established that 5,000 r ("delayed lethal dose") suffice to arrest any further multiplication of cells in tissue culture (7); on the other hand, many millions of r are required for complete inactivation of the neoplastic viruses (8, 11, 24, 41, 42). Irradiation appeared to be a particularly appropriate method for demonstrating the causative agent in tumor tissue because it involves no danger of retention of the agent by ultrafilters, and safeguards it in its original concentration. Besides, it is doubtless more reliable than freezing, since mammalian tumors have often been found readily transmissible after having been subjected to this treatment (3, 19-22, 25, 39), but their transmission is never successful after exposure to x-ray doses ranging from 1,000 to 6,000 r (1, 17, 29, 34, 40).

Our experiments reveal that the irradiation method makes possible the cell-free transfer of a number of Rous sarcomas that cannot be transferred with Berkefeld and paper-pulp-sand filtrates; yet cases remain in which no evidence for an agent separable from the cells can be found, even with the method of irradiation.

We should like to point out that this absence may be apparent rather than real. Considering the experiments of Murphy and his co-workers (26, 27), of Sittenfield, Johnson, and Jobling (37, 38), and of Claude (5), as well as of Carr (4), the possibility should not be disregarded that the transmitting agent may still be present in the irradiated material, its activity being counteracted by an associated inhibitor, or by an antibody. Furthermore, it may well be that the causative agent is present, though in such minute quantities that with our only test object, the susceptible fowl, its presence cannot be detected. Whether the observations made here have any possible bearing upon the significance of the nonfilterable phase of virus-induced fowl sarcomas is thus problematical.

SUMMARY

Five out of 10 slowly growing Rous fowl sarcomas were found to be nontransmissible by both Berkefeld and paper-pulp-sand filtrates. After irradiation with doses lethal for the sarcoma cells, but practically harmless to the causative agent, 3 out of these 5 tumors could be transmitted, while the remaining 2 could not. The significance of these findings for the understanding of the so-called nonfilterable phase of the fowl tumors is discussed.

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The Effect of Progesterone and Testosterone Propionate on the Incidence of Mammary Cancer in Mice*

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It has been variously reported (1, 2, 8, 11) that the incidence of spontaneous mammary adenocarcinoma in breeders of the RIII strain from 6 to 12 months old ranges between 54 and 90 per cent, and is about 37.3 per cent after the first 18 months. Haagensen and Randall (6) found that 52.1 per cent of virgin females and 71.5 per cent of bred females of the same strain developed mammary cancer. Twombly (17) states that mammary tumors occur in 74.4 per cent of virgin females of the RIII strain.

It has been found in this laboratory (8) that the incidence can be reduced to 19 per cent when young females of this strain are injected subcutaneously with testosterone propionate as a prophylactic measure. Similar results have been reported by Gardner (5), Jones (9), Nathanson and Andervont (15), and by Loeser (13).

Lipschütz (12) has reported that testosterone with progesterone inhibited the growth of fibromas of the guinea pig uterus; and Heiman (7), that this combination reduced the glandular components of benign mammary adenofibroma of the rat and lowered the percentage of takes from 66.6 to 8.3.

MATERIAL AND METHODS

It has been suggested that progesterone be given alone or with testosterone to females of the RIII strain under conditions similar to those obtaining when only testosterone was administered (8). Accordingly 96 RIII mice of a brother-to-sister bred strain were treated. Inasmuch as the incidence of mammary cancer in virgin or bred mice of this strain varied in different laboratories, the reproductive history was not ascertained. The animals were fed on one part Purina dog chow checkers and two parts Rockland rat diet, given water *ad libitum*, and observed until death. The females littered in the early months of treatment.

The experiments were begun in February, 1942, and continued to December, 1944. The accompanying tables set forth in detail the procedure and observations.

Twenty-four female mice (Table I, Boxes I and II) 6½ months old were given subcutaneous injections of progesterone between February 11, 1942 and June 9, 1942. The injections, each of 0.2 mgm. dissolved in 0.1 cc. of sterile peanut oil, were given alternately on the right and left sides. One animal, which had received a total of 0.6 mgm. of progesterone, developed a mammary carcinoma 3 weeks after injections were started. A second mouse developed a similar growth after 8 weeks, following 1.6 mgm. progesterone; a third, 11 weeks after the first injection and after 2.2

TABLE I: PROGESTERONE IN FEMALE RIII MICE

Box I	Age at beginning of treatment, days	Dose, mgm.	Survival, days	Tumor
TREATMENT EVERY 6 DAYS: 4 TO 18 INJECTIONS, EACH 0.2 MGM.				
1	195	0.8	215	+
2	195	2.0	257	0
3	195	2.0	257	0
4	195	2.0	257	0
5	195	2.2	265	0
6	195	2.4	271	+
7	195	2.6	277	0
8	195	3.4	301	0
9	195	3.6	312	0
10	195	3.6	342	0
11	195	3.6	376	0
12	195	3.6	415	0
TREATMENT EVERY 6 DAYS: 8 TO 18 INJECTIONS, EACH 0.2 MGM.				
Box II				
1	190	1.6	245	+
2	190	1.8	259	0
3	190	2.0	296	0
4	190	2.0	296	0
5	190	3.2	310	0
6	190	3.2	310	0
7	190	3.6	357	0
8	190	3.6	364	+
9	190	3.6	370	0
10	190	3.6	371	0
11	190	3.6	421	0
12	190	3.6	421	0

* Testosterone propionate for these experiments was generously furnished by the Schering Corporation; Roche-Organon, Incorporated; and Ciba Pharmaceutical Products, Incorporated; progesterone by Roche-Organon, Incorporated, and by E. R. Squibb and Sons.

mgm. A mammary tumor appeared in a fourth mouse 2 months after the injections had been completed.

Twenty-four young female mice (Table II, Boxes III and IV) 8 weeks old received 6 mgm. of progesterone in divided doses during 8 weeks (June 11, 1943, to August 9, 1943). The progesterone was dissolved in peanut oil in the proportion of 1 mgm. to 0.1 cc. of oil. The first death of a tumor-free mouse occurred at 7 months of age. The last death occurred at 20 months, and a tumor was found in the left axilla. Another mouse developed a perivulvar tumor at

TABLE II: PROGESTERONE IN FEMALE RIII MICE

TREATMENT EVERY 10 DAYS: 6 INJECTIONS, EACH 1.0 MGM.

Box III	Age at beginning of treatment, days	Dose, mgm.	Survival, days	Tumor
1	62	6.0	327	0
2	62	6.0	344	0
3	62	6.0	487	0
4	62	6.0	511	0
5	62	6.0	511	0
6	62	6.0	518	0
7	62	6.0	518	0
8	62	6.0	568	+
9	62	6.0	594	+
10	62	6.0	628	+
11	62	6.0	628	0
12	62	6.0	628	0
TREATMENT EVERY 10 DAYS: 6 INJECTIONS, EACH 1.0 MGM.				
Box IV				
1	55	6.0	231	0
2	55	6.0	231	0
3	55	6.0	375	0
4	55	6.0	494	0
5	55	6.0	494	0
6	55	6.0	501	0
7	55	6.0	501	0
8	55	6.0	551	0
9	55	6.0	551	0
10	55	6.0	551	0
11	55	6.0	577	0
12	55	6.0	611	+

18 months of age; a third showed a small growth in the left axilla; and a fourth in the left groin. These last 2 animals were over 20 months of age. Though the injections were given between the second and fourth months of life, the tumors appeared in these 5 mice more than 1 year after the progesterone was administered; yet in this strain 54 to 90 per cent of the females develop spontaneous mammary cancer at from 6 to 12 months of age, as has been said.

Thus, of 48 mice treated with progesterone alone only 8, or 16.6 per cent, developed mammary cancer, and in 4 instances the animals were over 1 year of age. The average survival period of the injected animals was more than 1 year, and 19 survived 14 months without the appearance of tumors.

Another series of 12 animals, 6 months old, received progesterone in doses of 0.2 mgm. in 0.1 cc. oil and 0.2 mgm. of testosterone propionate in 0.1 cc. oil, both every 3 days (Table III, Box III). One developed a tumor 9 weeks after the first injection was given and it had received 2 mgm. of progesterone and testosterone propionate. The second showed a tumor 5 months after injections were begun, having received 3.4 mgm. of progesterone and testosterone propionate.

A second series of 12 mice, 6 months old, received the same amount of progesterone and testosterone propionate in the same time (Table III, Box IV). The first tumor appeared 3 weeks after injections were started. Eleven animals dead between the tenth and 13th months of age were negative for tumors.

Twenty-four female mice, 8 to 11 weeks old, received weekly during 8 weeks 6 mgm. progesterone alternating with 6 mgm. of testosterone propionate, both dissolved in sterile peanut oil so that 1 mgm. was contained in 0.1 cc. (Table IV, Boxes VII and VIII). The first died at 5 months and the last at 20 months of age. No tumors appeared in these animals.

Thus of 48 mice treated with testosterone propionate and progesterone only 3, or 6.2 per cent, developed mammary cancer, as compared with 16.6 per cent in those treated with progesterone only. Microscopic examination of the 11 spontaneous carcinomas in the 96 mice of these 2 groups showed no variation from those appearing spontaneously in untreated control animals.

Thirty-six RIII females were spayed when 8 weeks old. Twelve received 7 mgm. of progesterone divided into 7 subcutaneous weekly injections. Twelve others were given 6 mgm. each of testosterone propionate and progesterone divided into 6 subcutaneous doses. Twelve were untreated. One of the spayed controls developed a sarcoma of the lower jaw, a spontaneous tumor rarely seen in this strain, perhaps as a result of trauma. None of the other treated or control mice in this group had tumors of any sort.

The effect of testosterone propionate and progesterone was then studied in 18 mice with transplanted malignant tumors. Six dba female mice were injected weekly with 6 mgm. of testosterone propionate and 6 mgm. of progesterone. Two days after the last injection subcutaneous implants of mammary carcinoma RC (Taylor) were made. Growths appeared in the 6 animals on the seventh day as usual.

Six C57 black females were similarly injected with the same hormones. Transplants of mammary carcinoma 755 (Bagg) introduced subcutaneously grew in all after the seventh day.

Sarcoma 180 grew in 6 female RIII mice into which

TABLE III: PROGESTERONE AND TESTOSTERONE PROPIONATE ALTERNATELY IN FEMALE RIII MICE

TREATMENT EVERY 3 DAYS: 4 TO 34 INJECTIONS, EACH 0.2 MGM.

Box III	Age at beginning of treatment, days	Progesterone, mgm.	Testosterone propionate, mgm.	Survival, days	Tumor
1	184	2.0	2.0	246	+
2	184	2.2	2.2	262	0
3	184	3.4	3.4	325	+
4	184	3.4	3.4	343	0
5	184	3.4	3.4	360	0
6	184	3.4	3.4	360	0
7	184	3.4	3.4	360	0
8	184	3.4	3.4	360	0
9	184	3.4	3.4	382	0
10	184	3.4	3.4	382	0
11	184	3.4	3.4	382	0
12	184	3.4	3.4	382	0
Box IV					
1	195	0.4	0.4	204	+
2	195	3.2	3.2	300	0
3	195	3.4	3.4	338	0
4	195	3.4	3.4	345	0
5	195	3.4	3.4	345	0
6	195	3.4	3.4	356	0
7	195	3.4	3.4	356	0
8	195	3.4	3.4	381	0
9	195	3.4	3.4	381	0
10	195	3.4	3.4	381	0
11	195	3.4	3.4	387	0
12	195	3.4	3.4	387	0

TABLE IV: PROGESTERONE AND TESTOSTERONE PROPIONATE IN FEMALE RIII MICE

TREATMENT EVERY 7 DAYS: 12 ALTERNATING INJECTIONS, EACH 1.0 MGM.

Box VII	Age at beginning of treatment, days	Progesterone, mgm.	Testosterone propionate, mgm.	Survival, days	Tumor
1	78	6.0	6.0	395	0
2	78	6.0	6.0	398	0
3	78	6.0	6.0	400	0
4	78	6.0	6.0	414	0
5	78	6.0	6.0	460	0
6	78	6.0	6.0	460	0
7	78	6.0	6.0	460	0
8	78	6.0	6.0	460	0
9	78	6.0	6.0	474	0
10	78	6.0	6.0	524	0
11	78	6.0	6.0	587	0
12	78	6.0	6.0	600	0
Box VIII					
1	58	6.0	6.0	142	0
2	58	6.0	6.0	234	0
3	58	6.0	6.0	234	0
4	58	6.0	6.0	348	0
5	58	6.0	6.0	367	0
6	58	6.0	6.0	433	0
7	58	6.0	6.0	480	0
8	58	6.0	6.0	502	0
9	58	6.0	6.0	513	0
10	58	6.0	6.0	555	0
11	58	6.0	6.0	555	0
12	58	6.0	6.0	555	0

it had been transplanted after similar hormonal injections.

The growth of these 3 transplantable tumors in animals that had received testosterone and progesterone proves that the hormones had no inhibiting effect on already established mammary cancer. This is further verified by the appearance of spontaneous mammary tumors in treated female mice shortly after a few injections had been given. Microscopic tumors were probably established before treatment was begun.

DISCUSSION

Histologic studies of mammary gland from animals treated with testosterone and progesterone revealed a few compressed alveoli and ducts surrounded by fibrotic connective tissue. The cells lining the acini and ducts were small, little secretion was seen in the lumina, and desquamation was evident. The nipples were flat and involuted.

The hormones employed probably reduced the pituitary gonadotropic fraction and this deficiency, in turn, was followed by a suppression of ovarian secretion. It is known that in cancer-susceptible strains of mice the ovarian estrogens may stimulate the mammary gland and act as one factor in the production of cancer.

The normal fluctuations in the functional activity of the gland during estrus and pregnancy were probably moderated by the male hormone administered in these experiments, so that circulatory changes and mammary engorgement were diminished, a neutralizing effect that has been described by Korenchevsky and Hall (10). It may be noted that sterility occurred in the animals receiving only testosterone (8); when it was given with progesterone, the mice littered, but had few young. It will be recalled that in 4 of the mice treated only with progesterone the tumors appeared about 1 year after the injections had been completed, when the indirect effect on the normal mammary tissue had probably disappeared. It is possible that this hormone in combination with testosterone prevents the initiation of mammary cancer in the Paris RIII strain by causing involution of the mammary tissue, as the histologic findings suggest, but the synergistic effect of the two hormones is obscure. The reduction in cancer incidence brought about by treatment with testosterone, progesterone, or both, is corroborated by the fact that spayed and treated females of this strain did not develop any mammary tumors, though both control and injected mice survived 16 to 24 months.

Those who criticize the use of testosterone in women for the prevention of mammary cancer have pointed out the danger of masculinization and sterility, but the small doses now employed in conjunction with progesterone have not been sufficient to produce these effects. Furthermore, the literature contains numerous reports on the beneficial action of testosterone propionate alone in large doses without any untoward results (3, 4, 14, 16).

It has been suggested in a previous article (8), therefore, that testosterone be given in prophylactic doses to women with a family history of cancer who complain of mammary disturbances. No untoward result has been noted in 16 women who have received these injections at the hands of the author for over 6 years, and who previously had complained of pain, swelling, and tenseness in the mammary glands. The synergistic effect of progesterone makes possible the reduction of testosterone.

CONCLUSION

1. Subcutaneous injections of progesterone in 48 brother-to-sister bred RIII female mice between the ages of 2 and 6 months reduced the incidence of spontaneous mammary adenocarcinoma from 54 per cent to 16.6 per cent.

2. Subcutaneous injections of testosterone propionate with progesterone further reduced the incidence to 6.25 per cent in 48 treated female mice between 2 and 6 months of age, the progesterone acting as an adjuvant.

3. Preliminary subcutaneous injections of these two hormones did not prevent the growth of transplanted mouse carcinoma and sarcoma.

4. Spayed females injected with testosterone and progesterone did not develop any mammary tumors. One control spayed female developed a sarcoma of the lower jaw, apparently unrelated to the experiment.

5. The injection of testosterone propionate and progesterone is suggested as a prophylactic measure in women with a family history of cancer.

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The Influence of Caloric Restriction and of Dietary Fat on Tumor Formation with Ultraviolet Radiation*

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There is considerable evidence that the appearance of neoplasms can be delayed and the incidence of tumors reduced in experimental animals when the total intake of food is restricted in amount (4, 5, 11-13, 19-22, 25, 27). This effect appears to be due largely to a general caloric restriction rather than to the lack of some specific growth factor and is especially significant since the animals on the curtailed rations are usually in better health and live longer than those allowed food *ad libitum* (12, 21). The effect of caloric restriction has been demonstrated for neoplastic diseases arising spontaneously in animals susceptible to lung adenomas (20), mammary tumors (21, 25, 27), and lymphoid leukemia (19), as well as for tumors induced by the use of carcinogenic hydrocarbons (11, 21, 22, 27).

In the present experiment an investigation was made of the effect of caloric restriction on the formation of tumors induced in mice by ultraviolet radiation (1). The influence of dietary fat on the development of the neoplasms was also studied. Previous studies (2) had shown that an increase in the fat content of the diet from 5 per cent to from 25 to 30 per cent accelerated the appearance of tumors due to ultraviolet irradiation. There is probably more than one explanation for the stimulating influence of fats. A direct local stimulation can be demonstrated by painting an oil on the ears of mice that are being irradiated (17). A film of oil on the skin forms a smooth surface that decreases the reflection of light and facilitates the penetration of the skin by radiant energy. Unless special precautions are taken, an oily film also forms on mice that are fed a diet high in fat, and in such animals the acceleration of carcinogenesis could result from the superficial effect of the fat (16).

Furthermore, the presence of fat in the diet also appears to have a more general effect on the animal, since mice on diets containing high levels of fat voluntarily ingest more calories than animals on the control diets (11). The present experiment was designed, therefore, not only to determine the caloric influence but also to detect any specific effect of dietary fat on the formation of tumors due to ultraviolet irradiation. Ultraviolet light is an ideal agent for studies of this nature, since there is no possibility of diet altering the primary carcinogen as could happen when azo dyes or hydrocarbons are administered, if precautions are taken to prevent oiliness of the skin.

MATERIALS AND METHODS

Young albino strain C male mice were used for the experiment. Groups of 24 were kept on shavings in metal box cages at all times except during irradiation, when they were placed in a special cage 25.5 cm. square and 3 cm. deep, constructed of wire mesh and divided into 24 individual compartments to prevent the mice from huddling together and to minimize their movements (18). In all cases the mice were irradiated for 30 minutes a day, 3 days a week, for 9 months with a medium pressure mercury vapor lamp. All groups received an intensity of approximately 2×10^4 ergs/cm^2/sec., resulting in a daily dose of about 3.6×10^7 ergs/cm^2.

The mice were divided into 4 groups of 48 each, and the different groups were given diets that varied in the levels of fat and calories supplied. The composition of each diet and the amounts fed daily are given in Table I. The mice in Groups 1 and 2 each received a calculated average of 6.7 calories per mouse per day, and Groups 3 and 4 of 9.7 calories per mouse per day. The daily allotment of food given each mouse in Groups 1 and 3 contained 45 mgm. of fat, whereas those in Groups 2 and 4 received 305 mgm. of fat daily.

The percentages of casein, salts, and vitamins were such that all groups received identical amounts of each of these ingredients daily. Variations in caloric value were accomplished by changing the amount of cerelose, by feeding different amounts of each ration, and by adding a proper amount of an inert cellulose

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product (celluration) to each diet. This latter substance is important also since it tends to hold the oil, imparting a less oily consistency to the diet and thus minimizing the complicating factor that results from the local effect of a film of oil that forms on the animals when greasy diets are employed.

The mice were fed once each day with a weighed quantity of food, the amount of which was determined from preliminary experiments. The diets were so adjusted that the widest possible difference in caloric intake between the groups on the restricted diets and those receiving the higher levels was obtained. For the conditions of this experiment, the amounts of food given represent approximately the minimum and health for the rest of the experiment. A small initial weight loss was observed in the mice on the higher caloric diets also, but this was entirely regained by the end of the first month.

The weight of the mice in Group 3 remained about constant throughout the experiment, whereas those in Group 4 gained slowly during the first few months; there was some weight loss in Group 4, however, during the last month. The mice on the high caloric diets remained in excellent health throughout the entire experiment, and at 5 months all the original mice were still alive. The mice on the restricted diets ate all their food ravenously within 5 or 6 hours after receiving it, and for much of the remainder of

TABLE I: COMPOSITION OF DIETS

Type of diet	1 Low calorie-low fat			2 Low calorie-moderate fat			3 High calorie-low fat			4 High calorie-moderate fat		
Constituents	Per cent	Gm./ mouse /day	Cal. /100 gm.	Per cent	Gm./ mouse /day	Cal. /100 gm.	Per cent	Gm./ mouse /day	Cal. /100 gm.	Per cent	Gm./ mouse /day	Cal. /100 gm.
Cerelose *	57.3	1.26	194.4	29.90	0.54	101.6	76.50	2.14	260.0	56.80	1.42	193.2
Casein	20	0.44	88.0	24.44	0.44	107.2	15.70	0.44	69.0	17.60	0.44	77.5
Corn oil	2	0.045	18.6	17.12	0.305	159.2	1.57	0.045	14.6	12.32	0.305	114.6
Salts	4	0.09	0	4.88	0.09	0	3.14	0.09	0	3.52	0.09	0
"Vitab" †	2	0.045	4.2	2.44	0.045	5.1	1.57	0.045	3.3	1.76	0.045	3.7
Celluration ‡	14.7	0.32	0	21.22	0.38	0	1.52	0.04	0	8.00	0.20	0
Total	100	2.2	305.2	100	1.8	373.1	100	2.8	346.9	100	2.5	389.0
Calories per mouse per day		6.7			6.7			9.7			9.7	

Each kilogram of diet also contained 80 mgm. of halibut liver oil.

* Cerelose—A pure glucose monohydrate containing 91% glucose. Obtained from the Corn Products Refining Company, New York, N. Y.

† "Vitab" rice bran concentrate. Carbohydrates compose 62% of "Vitab," a source of the B vitamins obtained from the National Oil Products Co., Harrison, N. J.

‡ A pure cellulose product prepared from rice hulls. Obtained from the Fischer Scientific Co., Pittsburgh, Pa.

Calories calculated on the following basis:

1 gm. casein = 4.4 cal.
1 gm. cerelose = 3.4 cal.
1 gm. corn oil = 9.3 cal.

See BODANSKY, M. Introduction to Physiological Chemistry. Fourth Edition. New York: John Wiley and Sons, Inc. 1938, p. 510.

the maximum amounts for a prolonged period. The mice were weighed every 2 weeks and carefully examined for the appearance of tumors; when neoplasms appeared the affected mice were removed from the experiment.

RESULTS

In general, the condition of the animals throughout the experiment remained good. This was true for the entire period except for the first month, during which the mice on the restricted diets lost an average of about 5 to 7 gm. per mouse. Eighteen mice from Group 1 and 10 mice from Group 2 died during this critical period. Thereafter, however, the survivors appeared to become adjusted to the low caloric intake; by the third month they had regained most of the weight previously lost, and they remained in excellent the day were very active in their search for more food. This increased activity was continued throughout the experiment, but appeared to decrease somewhat after the first few weeks. The gain in weight and the improved condition after the initial critical period could have resulted from a slowing-up in activity; or it might also have been due to the development of a more efficient type of metabolism, by means of which the mice utilized their food more effectively. Those that received the higher caloric diets required most of the day to consume the quantity of food given them, and were quiet most of the day. Except for the slightly heavier weight of the mice in group 4, these latter mice resembled stock mice fed dog biscuit *ad libitum*.

The irradiation produced an erythema in all the groups, but this was less in the mice maintained on

the restricted diets. Neoplasms of the ears were first observed at 6 months in Groups 2, 3, and 4. The rate of tumor formation was much higher in the groups that received the high caloric diets, and was most rapid in the group receiving the high calorie-low fat diet. Thus after 9 months 42 of a possible 48 mice (Group 3) had ear tumors (87 per cent), 30 of a possible 48 in Group 4 (63 per cent), whereas only 2 mice in Group 1 (7 per cent) and 9 in Group 2 (24 per cent) had neoplasms (Table II). The histological appearance of the tumors was similar to those described previously, and no difference was observed in the character of the neoplasms among the various groups.

TABLE II: THE INFLUENCE OF CALORIC RESTRICTION AND OF DIETARY FAT ON TUMOR FORMATION WITH ULTRAVIOLET IRRADIATION

	Diet	No. of mice (effectual total)	Weight of mice				Cumulative tumor count after first application of carcinogen, months					No. of mice tumor-free and alive at end of experiment	Per cent tumors (based on effectual total)
			Start of exper.	5 mos.	9 mos.	Av. wt. for entire exper.*	5	6	7	8	9		
1	Low cal.-low fat	29	22.4	19.2	19.5	18.8	0	0	0	1	2	27	7
2	Low cal.-moderate fat	38	22.6	21.0	21.2	20.4	0	2	4	7	9	28	24
3	High cal.-low fat	48	23.2	23.7	21.8	22.9	0	4	16	30	42	2	87
4	High cal.-moderate fat	48	23.0	28.1	26.0	27.2	0	3	9	22	30	17	63

* Average weight based on weights obtained every 2 weeks throughout the experiment. The lower average for groups 1 and 2 is largely the result of the lighter weight of the mice during the first 3 months.

DISCUSSION

Although several investigators have observed that underfeeding retards the development of tumors comparatively little attention has been given to the mechanism involved. However, some data are available concerning the period when the process of carcinogenesis is most susceptible to caloric restriction. There is considerable evidence that the development of a tumor occurs not as a single uninterrupted process, but rather in a series of steps (3, 7, 9, 23), and it has been suggested that the formation of cancer can be divided into 3 phases (9, 15): (a) the induction period, during which neoplastic cells are formed; (b) the critical or reversible period, which starts at the moment when the neoplastic cells are formed and continues until the neoplasm has become well established, and in which the neoplastic cell, or small nest of cells, is most susceptible to the environment; and (c) the period of progression, in which the neoplastic cells have gained ascendancy over the forces that hold them in control. This latter is the phase of relatively unrestricted invasive growth, during which regressions are infrequent.

Tannenbaum maintains that there is relatively little influence of dietary restriction or of high fat diets during the period when the tumor is being initiated (22, 24), or on the growth of established tumors (21), the stage we have called the period of progression. Lavik and Baumann (10) have shown that a high fat diet is most effective in accelerating tumor formation during the middle period of carcinogenesis; *e.g.*, 1½ to 3 months after the first application of methylcholanthrene. This might suggest that the primary effect of underfeeding or of diets high in fat is exerted during the critical period. Early in the critical stage the neoplasm consists merely of a nidus of relatively few cells. It has not yet established an independent blood supply, and the tumor cells must compete with the adjacent normal ones for the available food in the tissue fluids. If an abundant diet is eaten surplus energy would be available for the growth of tumor cells even after the ordinary bodily requirements had been met, whereas on a restricted diet the supply of nutritive elements would be limited to a degree that would adversely influence the proliferation of neoplastic cells. However, once the tumor attains a size sufficient to have its own blood supply it no longer needs to compete with the neighboring normal cells for available food in the tissue fluids. At such time the tumor enters the third stage of development, and the effect of dietary restriction on its subsequent growth is minimized.

It has been previously suggested that the action of fat is both local and systemic (8, 11, 21), and that the systemic effect is exerted largely through the mechanism of an increased consumption of calories. It now appears that under special circumstances there may also be a specific or residual effect of fat *per se*. Under experimental conditions in which it is possible for all three mechanisms to contribute to the end result, the rate of tumor formation may be increased considerably, and the incidence of tumors at 6 months can reach 86 per cent on a diet high in fat, as compared to only 17 per cent on a low fat diet (11), a difference of 69 per cent.

It has been demonstrated repeatedly that the ap-

plication of oil to the skin of mice painted with a hydrocarbon (8, 11, 14, 26), or irradiated with ultraviolet light (17), increases the rate of tumor formation without any change in diet. Mice fed diets high in fat often develop a greasy fur, and hence the increased tumor incidence observed could have been due in part to this local factor. That such is actually the case is indicated by experiments in which the local factor is minimized, (a) by a special feeder (11) that tends to prevent contact between the skin and the fat in the food; (b) by feeding fat emulsified in the drinking water (11); (c) by moulding moist crude diets into pellets (24); and (d) by adding an absorbent to the ration, as was done in the present experiments. Under these conditions the incidence of tumors at 6 months was 18 per cent on the low fat diet and 43 per cent on high fat (11, 24), but when the emulsion of fat was fed the incidence at 6 months was only 29 per cent (11). Thus the attenuation of the local factor lowers the effectiveness of the dietary fat, but does not prevent it entirely, and hence the non-local increment is considered to be due to a systemic influence.

When experimental animals are fed *ad libitum*, the systemic effect appears to be due in part to a voluntary increase in caloric intake by the mice fed the high fat diet; or conversely, to a reduced caloric intake by those on the low fat diet. The former usually consume about 15 per cent more calories per gram of mouse than the latter, and in some series the difference in caloric intake may be as much as 30 per cent on this basis (11). It follows, therefore, that no conclusions can be drawn as to the relative merits of caloric restriction or fat intake unless based upon experiments in which the caloric intake of the animals has been rigidly controlled.

When the caloric intake is equalized, and when contact between fat and the skin is eliminated as much as possible, there still appears to be a stimulatory effect of dietary fat on the formation of tumors. The increase is not very great when the caloric intake is high, but on low calorie diets the residual or specific effect of fat may be appreciable. Lavik and Baumann (11) fed 8 calories per 25 gram mouse daily for 6 months and observed 28 per cent of tumors due to methylcholanthrene when a high fat diet was fed, whereas none developed on a low fat diet. Survival on the latter diet, however, was poor. In the present study mice fed 6.7 calories daily stabilized their weights at 19 to 21 gm. average, and 24 per cent developed tumors due to ultraviolet light when the moderate fat diet was fed, as compared to only 7 per cent on the low fat diet (Table II). These experiments were continued for 9 months, during which survival was good. Further indication of a specific effect of fat is found in Table III. Mice that have been fed different diets on the basis of calories per animal may change in weight to such an extent that the imposed restrictions are obliterated when calculations are made on the basis of body weight. After equilibrium in weight was reached, the mice on the moderate fat-high calorie diet (Diet 4) were consuming approximately the same number of calories per gram of body weight as those on the low fat-low calorie diet (Diet 1), 0.36 cals./gm. for each group (Table III). Nevertheless, 63 per cent of the former group developed tumors as compared to 7 per cent of the latter.

Fat actually appeared to retard tumor formation when 9.7 calories were fed to each mouse daily, the final incidence of tumors being 63 per cent when a moderate level of fat was given, and 87 per cent on a low fat diet (Table II). However, the average weight for the mice on the moderate fat diets was 27.2 gm. in contrast to 22.9 gm. for those on the low fat diet. Hence, per gram of mouse, the mice on the moderate fat diet were receiving 15 per cent less calories than those on the low fat diet—0.36 calories and 0.42 calories, respectively (Table III). Such calculations, however, should not be taken too literally, for the extra increment of weight is probably accounted for largely by an increased supplement of fat, which might be expected to have an effect on metabolism different from the effect of added weight due to protein.

TABLE III: CALORIC RESTRICTION CALCULATED PER GRAM OF MOUSE

	Low calories		High calories	
	1 Low fat	2 Mod. fat	3 Low fat	4 Mod. fat
Average weight of mice for entire experiment (grams)	18.8	20.4	22.9	27.2
Calories fed per mouse daily	6.7	6.7	9.7	9.7
Calories fed per gram of mouse daily	0.36	0.33	0.42	0.36
Percent tumors (based on effectual total)	7	24	87	63

It will undoubtedly take more study before the mechanism is known by which fat *per se* increases tumor incidence on a restricted caloric intake. This residual effect of fat may, for example, be concerned with the efficiency of utilization of food energy. Forbes and Swift (6) have pointed out that the specific dynamic effect of the given nutrient depends upon the proportion of the other nutrients present in the diet. Conceivably, therefore, the proportion of fat and carbohydrate might determine how efficiently a given amount of dietary protein is utilized and, therefore, how much is left over for tumor growth.

SUMMARY

The influence of calories on the development of tumors due to ultraviolet irradiation was determined in strain C albino mice, and the effect of the fat in the diet and its relation to the caloric content was investigated at the same time. The amount of ultraviolet irradiation received by the mice was approximately 3.6×10^7 ergs/cm.^{2} daily. They were divided into 4 groups of 48 mice each. Two groups received a calculated 6.7 calories per mouse per day, and the other 2 an average of 9.7 calories per mouse per day. One group on each of the high and low calorie levels was given a low amount of fat in the diet, whereas in the 2 other groups a moderate amount was incorporated. At the end of 9 months the incidence of ear tumors in the 4 groups of mice was as follows: 87 per cent for the high calorie-low fat group, 63 per cent in the high calorie-moderate fat group, 24 per cent in the low calorie-moderate fat group, and 7 per cent in the low calorie-low fat group.

The data of this and of other experiments previously recorded suggest that most of the accelerating action of fat on tumor formation can be explained on the basis of an increased caloric intake, but that fat *per se* also appears to increase the rate of tumor formation. The latter effect is particularly evident when the total intake of calories is restricted.

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Abstracts

Reports of Research

The Initiating and Promoting Elements in Tumor Production. An Analysis of the Effects of Tar, Benzpyrene, and Methylcholanthrene on Rabbit Skin. FRIEDEWALD, W. F., and ROUS, P. [Rockefeller Inst., New York, N. Y.] *J. Exper. Med.*, **80**:101-126. 1944.

Although benzpyrene and methylcholanthrene produce neoplastic changes in rabbit epidermis almost as fast as tar does, they elicit visible tumors much later. The authors find this difference among the substances is due to their different abilities to promote cell multiplication. This promoting influence is much stronger for tar than for methylcholanthrene and benzpyrene. It was found that conditions or compounds that induce hyperplasia may cause tumor growth if latent tumor cells have been produced in the epidermis.—D. S.

The Determining Influence of Tar, Benzpyrene, and Methylcholanthrene on the Character of the Benign Tumors Induced Therewith in Rabbit Skin. FRIEDEWALD, W. F., and ROUS, P. [Rockefeller Inst., New York, N. Y.] *J. Exper. Med.*, **80**:127-144. 1944.

The benign tumors of rabbit skin produced by benzpyrene and methylcholanthrene are nearly all of the same types that are produced by tar, namely frill horns, papillomas, and carcinomatoids. However, owing to the different amounts of stimulation and to the different selective encouragement by the polycyclic hydrocarbons and the tar on the latent neoplastic cells, the proportion and frequency of the tumor types that arise on application of these substances differ. Tar produces mainly papillomas and carcinomatoids and seldom the frill horns, while benzpyrene and methylcholanthrene frequently produce the frill horns as well as papillomas and carcinomatoids. Also the polycyclic hydrocarbons give rise to sebaceous adenomas, while tar does not. This difference is due to the destruction of the cells of the sebaceous glands by tar, while the polycyclic hydrocarbons increase the number of gland cells.—D. S.

The Influence of Epidermal Cornification upon Carcinogenesis in Hairless Rats. HUEPER, W. C. [Warner Inst. for Therap. Research, New York, N. Y.] *Cancer Research*, **5**:331-336. 1945.

When 120 hereditarily hairless rats, from 50 to 255 days old, and 12 haired albino rats, 120 days old, were painted twice weekly with a 0.5% benzolic solution of methylcholanthrene for periods up to 11 months, only 1 of the hairless rats developed malignant lesions. Although precancerous epidermal changes were seen in other hairless rats, none of the haired rats exhibited any appreciable reactions. The highly cornified skin of the hairless rats appears to be more susceptible to chemical carcinogens than that of the normal haired rat. This relation is reversed in regard to the carcinogenic action of ultraviolet rays. The significance of these observations with respect to the concept of cocarcinogens is pointed out.—Author's abstract.

Influence of Unsaturated Dibasic Acids on the Induction of Skin Tumors by Chemical Carcinogens. CRABTREE, H. G. [Labs. of Imp. Cancer Research Fund, Mill Hill, England] *Cancer Research*, **5**:346-351. 1945.

Maleic and citraconic anhydrides greatly retard the carcinogenic action of 3,4-benzpyrene and 1,2,5,6-dibenzanthracene on mouse skin, and often prevent the emergence of tumors in a precancerous area of skin. Malonic acid, α-naphthoyl cyanide, and several (mainly lower aliphatic) aldehydes have little or no influence on the carcinogenic process. Evidence is given that the unsaturated dibasic acids combine with SH-containing cell constituents and that this action is specifically correlated with the power of inhibiting carcinogenesis. The hypothesis that a first stage of the action of chemical carcinogens is their fixation, through S linkages, to cellular enzymes, is discussed in the light of these observations.—Author's abstract.

The Carcinogenicity of *m'*-Methyl-*p*-Dimethylaminoazobenzene and of *p*-Monomethylaminoazobenzene. GIESE, J. E., MILLER, J. A., and BAUMANN, C. A. [Coll. of Agriculture, and Med. Sch., Univ. of Wisconsin, Madison, Wis.] *Cancer Research*, **5**:337-340. 1945.

m'-Methyl-*p*-dimethylaminoazobenzene proved to be the most potent carcinogenic azo dye hitherto reported for the liver of the rat. On equivalent concentrations of dye, rats fed the *m'*-methyl derivative invariably lost more weight, developed a more severe cirrhosis, and formed large hepatic tumors more rapidly than when *p*-dimethylaminoazobenzene was fed. When 0.048% of *m'*-methyl-*p*-dimethylaminoazobenzene (¾ molar equivalent of the usual 0.060% *p*-dimethylaminoazobenzene) was fed for 2½ months, the incidence of hepatic tumors 2 months later was 100%. *p*-Monomethylaminoazobenzene was at least as carcinogenic as *p*-dimethylaminoazobenzene. Actually more tumors developed when rations containing the monomethyl compound were fed *ad libitum* than when *p*-dimethylaminoazobenzene was fed, but the rats fed the monomethyl compound ate more food, and therefore also consumed more dye. In young rats *m'*-methyl-*p*-dimethylaminoazobenzene proved to be the most toxic of the 3 dyes.—Authors' abstract.

The Pathogenesis of Tumors of the Liver Produced by Butter Yellow. OPIE, E. L. [Rockefeller Inst., New York, N. Y.] *J. Exper. Med.*, **80**:231-246. 1944.

A detailed description is presented of the pathological changes that occur in the liver of rats after the administration of *p*-dimethylaminoazobenzene.—D. S.

Induction of Hepatomas in Mice by Repeated Oral Administration of Chloroform, with Observations on Sex Differences. ESCHENBRENNER, A. B. [Nat. Cancer. Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **5**:251-255. 1945.

The effects of repeated oral administration of various doses of chloroform were studied in strain A mice. All 7 females that had received amounts of chloroform sufficient to produce central necrosis of liver lobules eventually developed hepatic cirrhosis and hepatomas, while no such lesions were observed in 10 animals without liver necrosis. The resultant tumors were histologically indistinguishable from those produced by similar administration of carbon tetrachloride and were resistant to the necrotizing action of chloroform. Renal necrosis, which killed the animals, occurred in all male mice receiving amounts of chloroform sufficient to cause liver necrosis; thus none of these animals survived long enough to develop liver neoplasms.—R. A. H.

Porphyrin Excretion of Harderian Glands in Its Relation to Actinic Carcinogenesis in Hairless Rats. HUEPER, W. C., and FIGGE, F. H. J. [Warner Inst. for Therap. Research, New York, N. Y., and Univ. of Maryland Sch. of Med., Baltimore, Md.] *Cancer Research,* **5**:328-330. 1945.

The porphyrin incrustations about the nose that were induced consistently in 10 haired rats by a diet deficient in riboflavin were not induced so quickly, so consistently, or so abundantly in 29 hereditarily hypotrichotic rats. This difference was the result of a difference in porphyrin, which was reflected in the lower porphyrin content and output of the harderian glands of the hairless rat. The harderian glands of the haired rat contained approximately 4 times as much porphyrin as those of the hairless rat. Ultraviolet irradiation of the skin increased the porphyrin content of the harderian glands in haired and hairless rats, on adequate or riboflavin-deficient diets. These observations may be regarded as circumstantial evidence in support of the hypothesis that porphyrin metabolism associated with the excretion of relatively large amounts of porphyrin by the harderian glands is one of the factors that influence susceptibility to light-induced cancer in rats.—Authors' summary.

Light as a Factor in Skin Cancer. WEISS, R. S., and CONRAD, A. H., SR. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America,* **24**:1028-1032. 1944.

The authors consider it probable that the sun's rays are the precipitating but not the ultimate cause of skin cancer. Animal experimentation gives considerable support to the theory that rays in certain regions of the spectrum may produce cancer. Although it is not permissible to apply these findings directly to man, it is justifiable to assume that since the wave lengths that produce animal tumors are the same as those principally affecting human skin, these wave lengths play a role in cutaneous cancer in man.—J. L. M.

Differential Tissue Response to Neutron and Roentgen Radiations. LAMPE, I., and HODGES, F. J. [Univ. of Michigan, and Univ. Hosp., Ann Arbor, Mich.] *Radiology,* **41**:344-350. 1943.

Experimental work on rabbit testis showed that a single massive dose of x-ray that will produce permanent sterilization cannot be given without producing permanent damage to the skin. When neutrons were used under the same biological conditions there was sufficient tissue selectivity within a restricted dosage range to produce sterilization without serious skin damage. These experiments suggest that neutron radiation may be more advantageous than roentgen radiation in the treatment of neoplasms. —R. E. S.

Beeinflussung des Eisenstoffwechsels durch Röntgen- und Radiumstrahlen mit besonderer Berücksichtigung der Verhaltnisse beim Collumcarcinom. [The Effect of Roentgen and Radium Irradiation on Iron Metabolism, with Special Reference to Cancer of the Cervix.] SCHUCK, J. [Munich Univ., Munich, Germany] *Arch. f. Gynäk.,* **172**:521-546. 1942. From abstr. in *Chem. Zentralbl.,* **II**:1381. 1943.

The iron content of the serum of 44 patients with carcinoma of the cervix was 30% below the average value for normal women. No increased storage of iron could be detected histologically in the tumors. After large doses of radium the serum iron content generally increased by a small amount, and after adjuvant x-ray therapy there was a further increase by about one-fourth of the original value. Three months after combined radiation therapy, the serum iron was generally above normal. An analogous increase was observed in a case of myoma treated in the same way. In 4 rabbits, x-radiation increased the serum iron. The increase of serum iron observed after the use of electromagnetic radiation is attributable not only to blood destruction but also to reticuloendothelium injury.—M. H. P.

La Fluorescence de la Peau Normale, des Lésions Cutanées et des Phanères. Sa Relation avec la Présence de Kératine et de Lipides. [The Fluorescence of Normal Skin, of Cutaneous Lesions, and of Skin Derivatives. Its Relation to the Presence of Keratin and Lipids.] DE CASTRO, M., and DE ALMEIDA, O. [São-Paulo Univ., São-Paulo, Brazil] *Rev. canad. de biol.,* **2**:361-377. 1943.

The degree of fluorescence observed with an ultraviolet lamp provided with a Wood's filter in pathologic lesions of the skin and mucosa, as well as in skin derivatives of man and the lower animals, is directly proportional to the quantity of keratin (and not of cholesterol or other lipids) present.

Not all skin lesions showing hyperkeratosis or squamae can be distinguished from one another with the aid of a Wood's filter. This method was of no diagnostic or prognostic value in the 27 cases studied, which included cancer, pemphigus foliaceus, psoriasis, eczema, Duhring's disease, lupus erythematosus, and so forth. It is also impossible to distinguish these pathologic lesions by fluorescence, from a nail, a white hair, a horn, or a mere cutaneous corn.—C. A.

Fluorescence Studies Related to the Cancer Problem. SIMPSON, W. L. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America,* **24**:1245-1254. 1944.

A review, with 37 references, of applications of fluorescence studies to the clinical and experimental investigation

of cancer. For diagnosis of various types of cancerous and precancerous lesions, some investigators have reported good results with macroscopic and microscopic examination under filtered ultraviolet light; photographs from 3 illustrative cases are presented. Fluorescence studies upon serum and urine have proved diagnostically inconclusive. In experimental investigations, fluorescence analysis has been useful in the identification and determination of carcinogens *in vivo* and *in vitro*.—J. L. M.

Degradation of Cystine Peptides by Tissues. III. Absence of Exocystine Desulfurase and Dehydropeptidase in Tumors. GREENSTEIN, J. P., and LEUTHARDT, F. M. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:249. 1945.

Exocystine desulfurase and dehydropeptidase were found to be absent from 12 additional tumors of mice, rats, guinea pigs, and rabbits. These results extend the original observations on mouse tumors (*J. Nat. Cancer Inst.*, **5**:39. 1944; abstr. in *Cancer Research*, **5**:57. 1945).—R. A. H.

Source of Tumor Proteins. I. Effect of a Low-Nitrogen Diet on the Establishment and Growth of a Transplanted Tumor. WHITE, F. R., and BELKIN, M. [Nat. Cancer Inst., Bethesda, Md., and Yale Univ. Sch. of Med., New Haven, Conn.] *J. Nat. Cancer Inst.*, **5**:261-263. 1945.

It was found that a transplanted mammary adenocarcinoma became established as well in mice fed a diet very low in protein as in mice with an adequate protein intake, although its growth rate was somewhat retarded by the dietary protein restriction. This was interpreted as indicating that the carcinomatous tissue could utilize for its metabolic needs the body proteins of the host.—R. A. H.

Source of Tumor Proteins. II. Nitrogen-Balance Studies of Tumor-Bearing Mice Fed a Low-Nitrogen Diet. WHITE, F. R. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:265-268. 1945.

Nitrogen-balance studies on mice bearing a transplanted mammary adenocarcinoma and placed on a diet of very low nitrogen content showed that the tumor was able to grow and thus increase in protein while the host animal was in negative nitrogen balance. This would indicate, in all probability, that the tissues of the host can supply the tumor with nitrogenous substances for growth. —R. A. H.

Einfluss von Sexualhormonen auf den Brenztraubensäure- und Zucker-Gehalt des Blutes von normalen und sarkomtragenden Ratten. I. [Influence of Sex Hormones on the Pyruvic Acid and Sugar Content of the Blood of Normal and Sarcoma-Bearing Rats. I.] v. EULER, H., SÄBERG, I., and HÖGBERG, B. [Vitamin Inst., Stockholm Univ., Stockholm, Sweden] *Ztschr. f. physiol. Chem.*, **268**:171-178. 1941.

In male rats bearing the Jensen sarcoma the pyruvic acid level of the blood was 3 to 4 times higher than that in control males. In several instances the pyruvic acid content decreased to the normal level about 10 days after surgical removal of the sarcoma. The injection of 50 or 100 γ of estrone into rats bearing the Jensen sarcoma led to a decrease in pyruvic acid level in the blood to about one-half the high value within 2 days. Testosterone propionate, when injected in 0.5 mgm. doses into sarcoma-bearing male rats, had a similar effect. The injection of 2.5 mgm. of testosterone propionate into normal rats during estrus also produced a decline in pyruvate level in the blood within 3 to 24 hours after injection.—K. G. S.

The Incidence of Adrenal Cortical Carcinoma in Gonadectomized Female Mice of the Extreme Dilution Strain. III. Observations on the Adrenal Glands and Accessory Sex Organs in Mice 13 to 24 Months of Age. WOOLLEY, G. W., and LITTLE, C. C. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] *Cancer Research*, **5**:321-327. 1945.

Two groups of mice of the ce strain 13 to 24 months of age were autopsied at monthly intervals and studied. The groups consisted of virgin females: (a) 15 that were unspayed, and (b) 41 gonadectomized. Ovariectomy was performed when the mice were 1 to 3 days of age. Adrenal cortical carcinomas were found in 100% of the ovariectomized mice and in none of the intact mice. Metastases to the lungs were observed in 9 mice. The condition of the accessory sex organs of the ovariectomized mice indicated that they were being subjected to influences similar to sex hormones. Variation in the amount and nature of these hormones was indicated.—Authors' abstract.

Studies on the Mammary Tumors of Dogs. Lactation and the Influence of Ovariectomy and Suprarenalectomy Thereon. HUGGINS, C., and MOULDER, P. V. [Univ. of Chicago, Chicago, Ill.] *J. Exper. Med.*, **80**:441-454. 1944.

The spontaneous mammary tumors of dogs were found to be of 3 types, a diffuse epithelial and connective tissue overgrowth, solid masses of epithelial cells, and intracystic papillomatous tumors. It was found that all the dogs with mammary tumors had suprarenal cortical tumors.

The milk secreted from the mammary tumors contained different amounts of solutes from those of normal milk.

Excision of the ovaries of normal dogs and of dogs with mammary tumors resulted, in some dogs, in a cessation of lactation even under the stimulus of prolactin, while in other dogs the ability of the breast to lactate was not abolished. The animals that were able to lactate after ovariectomy included all dogs with mammary tumors, all with pseudopregnancy, and certain other dogs that were presumably normal. However, even in dogs with breast tumors, lactation and the capacity to lactate when stimulated with prolactin did not continue indefinitely. Removal of the suprarenal glands did not usually modify lactation in dogs with prolonged lactation after the removal of the ovaries.—D. S.

Gene-Milk Agent Relationship in Mammary-Tumor Development. HESTON, W. E., DERINGER, M. K., and ANDERVONT, H. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:289-307. 1945.

By back-crossing $(C3H \times B)F_1$ hybrid female mice to C57 black and C3H males 2 groups of mice, exposed to identical maternal influences but of different genetic constitution, were obtained. Comparison of the incidence of mammary tumors in these 2 groups, as well as similar data from the second backcross generation, demonstrated

the influence of heredity upon mammary carcinogenesis. When these 2 groups of animals were used as foster parents for $(B \times C3H)F_1$ females (these animals lack the milk influence), the test animals nursed by $(C3HB \times B)BC$ females had a significantly lower mammary tumor incidence than did those nursed by $(C3HB \times C3H)BC$ foster mothers. This difference was interpreted as indicating that the genetic constitution of a mouse can determine its ability to propagate and transmit the milk influence. Analysis of the tumor incidences of fostered offspring of individual $(C3HB \times B)BC$ females substantiated this hypothesis. It was found that whereas those test animals fostered to some of the nontumorous females had a high incidence of mammary tumors, those fostered to others had a low or even a zero incidence of carcinoma, indicating a segregation of genes responsible for milk influence propagation in the backcross females. From these and other data it was suggested that multiple factors are involved in determining the ability of an animal to propagate the milk influence and that this ability and susceptibility to mammary carcinoma are governed by separate sets of genes.—R. A. H.

Milk-Induced Mammary Carcinoma in Mice. Haagensen, C. D., and Randall, H. T. [Coll. of Physicians & Surgeons, Columbia Univ., and Presbyterian Hosp., New York, N. Y.] *Cancer Research,* **5**:352-355. 1945.

Foster nursing induced mammary carcinoma in 76% of the females of a strain of mice (C57 black) in which there were no mammary carcinomas among the control animals. By a method of artificial feeding it was shown that as little as 0.2 cc. of milk is sufficient to induce mammary carcinoma. The carcinomas thus induced appear at a considerably later period in the life of the mouse than is usual in high mammary carcinoma strains. —Authors' abstract.

Metabolic Studies on Leukemic Mice with the Aid of Radioactive Phosphorus. Scott, K. G. [Univ. of California, Berkeley, Calif.] *Cancer Research,* **5**:365-367. 1945.

A comparison was made of the uptake of P^{32} by normal and lymphomatous mice and the subsequent retention and tissue distribution. Tumor tissue took up more P^{32} than did the lymph node controls. The uptake of P^{32} by tumors per mgm. of tissue phosphorus was greater than that by other tissues.—Author's abstract.

The Constitution of Mitochondria and Microsomes, and the Distribution of Nucleic Acid in the Cytoplasm of a Leukemic Cell. Claude, A. [Rockefeller Inst., New York, N. Y.] *J. Exper. Med.,* **80**:19-29. 1944.

The cytoplasmic constituents of leukemic cells were separated into 3 main fractions by differential centrifugation. The fractions were mitochondria, microsomes, and a third portion containing substances of relatively low molecular weight. Phosphorus distribution among the various fractions, and light absorption in the ultraviolet suggest that ribose nucleic acid occurs in the cytoplasm of leukemic cells only in association with formed elements of relatively large size, namely, microsomes and mitochondria.—D. S.

Certain Conditions Determining Enhanced Infection with the Rabbit Papilloma Virus. Friedewald, W. F. [Rockefeller Inst., New York, N. Y.] *J. Exper. Med.,* **80**:65-76. 1944.

It was previously shown by the author (*J. Exper. Med.,* **75**:197. 1942; abstr. in *Cancer Research,* **3**:556. 1943) that rendering a tissue hyperplastic before inoculation increased the effective titer of the Shope papilloma virus 10- to 100-fold. It was found in the present investigation that the effective titer was enhanced 10 to 100 times further by the protection of the scarified and inoculated area with a layer of paraffined gauze until healing occurred. The increase in titer resulting from the added procedure is attributed to the fact that the regeneration of the epithelial tissue is favored and that necrosis and subsequent formation of a scab is largely prevented; consequently the virus is in contact with the susceptible cells and not lost in the necrotic tissue.—D. S.

Studies on the Variation of the Rous Sarcoma Virus Following Growth of the Tumor in the Anterior Chamber of the Guinea Pig Eye. Shrigley, E. W., Greene, H. S. N., and Duran-Reynals, F. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research,* **5**:356-364. 1945.

The Rous sarcoma was grown in the anterior chamber of the guinea pig eye. Its behavior differed from that of mammalian tumors in a similar environment in that vascularization occurred in 48 to 72 hours after inoculation and that the transplant increased in size by 2 or 3 diameters in 2 weeks, after which time it remained quiescent for as long as 6 months. Evidence that the virus as well as the tumor survived guinea pig transfer was given by the fact that chickens inoculated with the transplant from the guinea pigs developed hemorrhagic disease as well as tumors.

Growth of the Rous sarcoma in the guinea pig resulted in an alteration in the properties of the virus for, when it was inoculated into chickens, periosteal tumors developed. Further, hemorrhagic disease occurred in tissues not frequently affected by the stock Rous virus, and finally, there was a suggestion of an alteration of species affinity for the chicken by one variant Rous strain. Since the 3 strains of the modified Rous virus studied showed different properties, it was evident that although the Rous sarcoma was grown in relatively the same environment in the guinea pig, the virus varied in different ways. It is considered that these present changes in the virus represent modifications that fall between those occurring after turkey-guinea fowl passages and the duck passages as previously described.—Authors' abstract.

Antigene and bösartige Geschwülste. [Antigens and Malignant Tumors.] Micheel, F., and Emde, H. [Münster Univ., Münster, Germany] *Ztschr. f. physiol. Chem.,* **266**:249-256. 1940.

The basis of these experiments was the hypothesis that the autonomous growth of malignant tumors may be related to anomalies in protein synthesis and breakdown, in such a way that the efficiency of the proteolytic enzymes of the normal cells is inferior to that of the tumor tissue thus making possible the destructive growth of the latter at the expense of normal tissues. Since previ-

ous *in vitro* experiments had failed to demonstrate fundamental differences between the proteolytic systems of normal and tumor cells, the authors placed the proteolytic enzyme system of the organism under strain by forcing it continuously to produce antibodies against strongly antigenic foreign proteins, *e. g.,* snake venoms, horse serum albumin, egg albumin. The test consisted in studying the development of benzpyrene tumors in albino mice while minute amounts of the antigens were administered continuously by the parenteral route. The observations indicate that the production of sarcoma after subcutaneous injection of 2 mgm. benzpyrene in olive oil is retarded and in some instances even prevented by frequent injections of the protein antigens in amounts ranging from 2 to 30 γ per dose. The authors stress the preliminary character of these findings.—See also *Ztschr. f. physiol. Chem.,* **269**:217-226. 1941; **275**:215-216; 258-266. 1942; abstr. in *Cancer Research,* **4**:718. 1944.—K. G. S.

Einige Beobachtungen über das Wachstum des Mäuse-Ascites-Tumors und seine Beeinflussung. [Observations on the Development of the Mouse Ascites Tumor and Factors Affecting It.] Lettré, H. [Allg. Inst. Geschwulstkr., Rudolf Virchow-Krankenhaus, Berlin, Germany] *Ztschr. f. physiol. Chem.,* **268**:59-76. 1941; **Addendum.** *Ibid.,* **271**:190-191. 1941.

When mice are injected intraperitoneally with suspensions of cells of the Ehrlich mouse carcinoma, the tumor cells may multiply diffusely throughout the peritoneal cavity, which simultaneously is filling up with ascitic fluid. The resistance of mice against the ascites-type Ehrlich tumor appears to be much less than that against other transplantable tumors; *i. e.,* the number of "takes" is much greater. Furthermore, test substances injected into the peritoneum of mice bearing the ascites tumor come into contact with the tumor cells better than do substances injected at a distance from a tumor in animals bearing solid tumors. The growth of the ascites tumor may be followed by plotting the weight curves of the animals. Substances found to interfere with the development of the ascites tumor were 5,6-benzo-9-phenyl-flavin and colchicine; neither physiological salt solution nor Prontosil solubile had such an effect.—See also *Naturwissenschaften,* **31**:467-468. 1943; abstr. in *Cancer Research,* **4**:719. 1944.—K. G. S.

Factors in Cancer Production. Cowdry, E. V. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America,* **24**:985-993. 1944.

A general discussion covering: external carcinogens (sunlight, x-rays, radium, coal tar products, petroleum oil, aniline dyes, burns, mechanical trauma); precancerous lesions; internal carcinogens (steroid compounds, *e. g.,* estrogens and bile constituents); viruses; heredity; milk influence; and accelerators and inhibitors of cancer in mice.—J. L. M.

Biological Differentiation of Benign and Malignant Growths. Greene, H. S. N. [Yale Univ. Sch. of Med., New Haven, Conn.] *Bull. New York Acad. Med.,* **20**: 595-598. 1944.

A review, with bibliography, of the author's studies in this field.—E. E. S.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

Diagnosis—General

Serologische Krebsdiagnose mittels proteolytischer Systeme. [The Serologic Diagnosis of Cancer by Means of Proteolytic Systems.] Podroužek, W. [Prague, Czechoslovakia] *Ztschr. f. Krebsforsch.,* **53**:185-207. 1942. From abstr. in *Chem. Zentralbl.,* **1**:1377. 1943.

A modification of the Fuchs carcinoma reaction is described that differs from the original in that 2 genetically paired carcinoma substrates are used, *i. e.,* carcinoma blood fibrin substrate and a carcinoma tissue substrate. Proteolysis is detected by a modification of the Coli method of Wollman. The accuracy was 97.6% in known cases of gynecologic cancer. Healthy persons without evidence of malignant neoplastic disease gave 92% negative results.—M. H. P.

Therapy—General

Castration for Advanced Malignant Growth: Short Historical Review with a Case Report. Howes, W. E. [Brooklyn, N. Y.] *Radiology,* **43**:272-274. 1944.

A case of melanoma of the conjunctiva of the eye was treated by radical surgery. Subsequent metastases in the skin, liver, and exenterated orbit were treated by castration with no beneficial results. Literature on the use of castration for cancer of the prostate, breast, and choroid, and for metastases, is briefly reviewed.—R. E. S.

Oestrogenic Substances Showing Anti-tumour Action. Tadros, W. [Fouad I Univ., Cairo, Egypt] *Nature, London,* **155**:366-367. 1945.

Oral administration of αα-di-(*p*-ethoxyphenyl)-β-phenylbromoethylene (100 to 600 mgm. daily) was followed by (1) disappearance of edema and relief of biliary obstruction in a case of recurrent mammary carcinoma with metastases in the liver; and (2) regression of an inoperable ovarian sarcoma.—E. L. K.

Skin and Subcutaneous Tissues

A Study of 106 Cases of Multiple Primary Skin Cancer. Cooper, Z. K. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America,* **24**:1022-1027. 1944.

During a 5 year period (1936 to 1941) a total of 1,790 cases of cancer of the skin were seen, and in 106, or 5.9%, the patient had multiple primary lesions confirmed by biopsy. All patients with multiple skin cancer in the group reported here were white. Cases of lesions of the lower lip, penis, vulva, and anus were omitted from this study, as were also cases of melanoma, in which satellite lesions and multiple skin recurrences and metastases are the rule although there is usually only one primary lesion.

Multiple skin cancer does not differ from solitary cutaneous cancer in sex or age incidence, or in location or

histologic type of the lesions. The attributes determining the production of multiple lesions in one type of skin and not in another under apparently similar environmental conditions are unknown, though susceptibility to the action of sunlight may be one factor operating in such cases.—J. L. M.

Two Cases of Multiple Arsenical Cutaneous Carcinoma. Russell, B. F., and Klaber, R. *Proc. Roy. Soc. Med.*, **38**:128-129. 1945.

Both patients received liquor arsenicalis (liquor potassii arsenitis U. S. P.) with bromides for epilepsy over long periods:

Case 1.—From 1912 to 1924 (total intake about 22 gm. As_2O_3 in 12 years) and during 1 month in 1935. In 1944 an ulcer developed on the thorax, which was excised and found to be a squamous carcinoma. No palmar or plantar keratoses were present.

Case 2.—From 1913 to 1943 (total intake about 47 gm. As_2O_3 in 31 years). In 1944, there were (a) multiple "plaques" on trunk and thighs, (b) early palmar keratotic changes, (c) a lesion on the nipple that showed "multiple basal-celled carcinoma with marked cystic degeneration."

There is no legal obstacle to the continued supply of arsenic that has once been prescribed by a medical practitioner. In the discussion, a case of carcinoma of the lung in a man who took large amounts of liquor arsenicalis for attacks of dermatitis herpetiformis was mentioned. —E. L. K.

Tragedy of Malignant Melanoma. Tod, M. C. [Holt Radium Inst., Manchester, England] *Lancet*, **247**:532-534. 1944.

Incompetent treatment of pigmented moles entails a danger of inducing malignancy in lesions that appear benign. Of 100 patients with malignant melanoma, observed at the Holt Radium Institute, 34 died as a direct result of incorrect treatment by simple excision, ligation of the pedicle, or chemical cauterization. It is advised that treatment of pigmented moles for cosmetic reasons should not be carried out unless such treatment is as radical as that adopted when signs of active growth have been observed. The treatment recommended for various types of malignant melanoma consists in radical surgery with block dissection of regional lymph nodes, radical radiation, or a combination of both. Four clinical photographs but no photomicrographs are incorporated in the article.—L. W. P.

Malignant Melanoma. Wigley, J. E. M., and Brain, R. T. [London, England] Letters to the Editor. *Lancet*, **247**:707. 1944; Brailsford, J. F. [Edgbaston, Birmingham, England] *ibid.*, **248**:34-35. 1945; Ellis, F. [London Hosp., London, England] *ibid.*, **248**:35. 1945; Denholm-Young, H. M. [Farningham, Kent, England] *ibid.*, **248**:129. 1945.

The first 2 of these letters criticize the article by M. C. Tod on "Tragedy of Malignant Melanoma," which appeared in *Lancet*, **247**:532. 1944 [see preceding abstract], on medical, statistical, and sociological grounds, and the third and fourth letters defend the same article. Wigley and Brain object to the proposal of irradiation as an alternative to surgery. Ellis, however, asserts that many malignant melanomas are radiosensitive [*Brit. J. Radiol.*, **12**:327. 1939].—M. H. P.

Disorders of the Liver and Extrahepatic Biliary Ducts Associated with Cutaneous Xanthomas and Hyperlipemia. Eusterman, G. B., and Montgomery, H. [Mayo Clin., Rochester, Minn.] *Gastroenterology*, **3**:275-286. 1944.

The association of cutaneous xanthomas and biliary cirrhosis of the liver in a woman of 48 is described. The hepatic disease is regarded as primary although there was no evidence of biliary retention on postmortem examination and all the bile ducts were freely patent. The patient had an extremely high plasma lipid content during part of her illness. Other cases of similar pattern are briefly mentioned. Complete explanation of the syndrome cannot be offered.—E. E. S.

Bowen's Disease, an Intra-Epidermal Carcinoma. Herold, W. C., and Cooper, Z. K. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America*, **24**:1033-1043. 1944.

In the 6 years from 1938 to 1944, there were 8 microscopically proved cases of Bowen's disease seen in the Barnard Clinic. This group comprised 0.3% of the 2,449 cases of carcinoma of the skin (basal, squamous, and basosquamous type) examined microscopically. This total figure does not include lesions of the lower lip, vulva, penis, or anus. The 8 case histories are presented, and the following points discussed: sex, race, and age incidence; occupation; family and personal history; duration of the disease; differential diagnosis; and treatment. Total excision of the tumor is recommended, by whichever method is best suited to the individual case: either scalpel excision, actual cautery, or surgical diathermy.—J. L. M.

Eye

Epithelial Tumors of the Iris. Asbury, M. K. [Cincinnati, Ohio] *Am. J. Ophth.*, **27**:1094-1106. 1944.

A candidate's thesis for membership in the American Ophthalmological Society. Cases of malignant and benign tumors from the literature, and previously unpublished instances, some from the Army Medical Museum, are described with an extensive bibliography. The 4 cases presented in most detail, with photographs and photomicrographs, were of relatively benign epithelial tumors affecting the iris principally and showing local destructiveness without producing metastases. They were all treated by enucleation.—E. C. R.

Capillary Hemangioma of Palpebral Conjunctiva. Wolfe, O. D. [Fort Riley, Kans.] *Am. J. Ophth.*, **27**:1289-1291. 1944.

Report of a case.—E. C. R.

Female Genital Tract

Theca-Cell Tumors of the Ovary. McGoldrick, J. L., and Lapp, W. A. [Kings Co. Hosp., Brooklyn, N. Y.] *Am. J. Obst. & Gynec.*, **48**:409-416. 1944.

Four cases of benign theca-cell tumor of the ovary are reported.—A. K.

Arrhenoblastoma of the Ovary. JOHNSON, C. G. [Tulane Univ. Sch. of Med., and Charity Hosp., New Orleans, La.] *Am. J. Obst. & Gynec.*, **48**:724-728. 1944.

A case report. Masculine characteristics had been present for 9 years before operation at the age of 24. After removal of the ovary bearing the tumor the patient became more feminine but retained some of the male secondary sex characters. A progestational endometrium was present. The opposite ovary, which also was removed, showed a dermoid cyst, circumstantial evidence in favor of the teratomatous origin of the arrhenoblastoma.—A. K.

Carcinoma of the Body of the Uterus. MORRIN, K. C. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America*, **24**: 1179-1184. 1944.

"This report is based upon an analysis of 88 cases of carcinoma of the uterus treated at the Barnard Free Skin and Cancer Hospital between the years 1919 and 1936. It is estimated that carcinoma of the uterus is responsible for 30% of all deaths from gynecological disease. Of these, over 10% are due to carcinoma of the uterine body.

"The poorest results were in the cases with cervical involvement, although a majority of these were not advanced cases. In the inoperable group, no patient survived 10 years, 20% survived 5 years, and 25% showed no evidence of disease 3 years after treatment. In the operable group a 10 year survival rate of 67.7% was obtained. Improvement in results can be chiefly effected by earlier diagnosis."—J. L. M.

The Diagnosis and Treatment of Cervical Cancer. EMMERT, F. V., and CLARKE, H. M. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *S. Clin. North America*, **24**:1185-1197. 1944.

Experience with approximately 1,000 cases of carcinoma of the cervix at Barnard Free Skin and Cancer Hospital over a period of 15 years forms the basis of a study on present-day methods of diagnosis and treatment of this widespread disease. The condition accounts for more than 80% of the hospital admissions on the gynecological service.

"The treatment of cancer of the cervix leaves much to be desired, except in early cases. . . . While the immediate mortality following radiation treatment is considerably less than that following hysterectomy, the late complications often leading to death are definitely more frequent. In stage I cancers, radical surgery in patients who are good operative risks presents definite advantages over radiation. In stage II cancers, radical surgery gives too high a primary mortality, hence radiation of the primary lesion is advisable. The authors are not prepared to say at this time whether radiation combined with the removal of the individual iliac glands will increase the survival rate. In stage III and stage IV cancers, radiation is the only means of treatment."—J. L. M.

Carcinoma of the Vulva. LEVIN, S. S., and CLARKE, H. M. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *S. Clin. North America*, **24**:1172-1178. 1944.

Of 63 women with carcinoma of the vulva seen during the years 1925 to 1940 at the Barnard Free Skin and Cancer Hospital, 41.2% had a history of leukoplakia of that region. The treatment of choice for vulval carcinoma is radical excision of the vulva and the inguinal lymph nodes. The rate of 5 year survival without evidence of disease was 45% among 40 patients treated by radical vulvectomy, and 31% among 13 treated by simple vulvectomy. Among the 40 patients of the former group, 15 and 7 showed unilateral and bilateral lymph node involvement, with 5 year, disease-free survival rates of 40 and 14.2%, respectively.—J. L. M.

MALE GENITAL TRACT

The Excretion of 17-Ketosteroids in Carcinoma of the Prostate. FRAME, E. G., and JEWETT, H. J. [Johns Hopkins Hosp., Baltimore, Md.] *J. Urol.*, **52**:330-333. 1944.

There was no difference in the total, α-, or β-17-ketosteroid excretion between a group of 16 patients with carcinoma of the prostate and a control group of 8 persons of similar age, nor between castrated and uncastrated patients in the former group. Excretion was decreased in men older than 40 years.—V. F. M.

Treatment of Prostatic Carcinoma by Castration and by Administration of Estrogenic Hormone: Comparison of Clinical Response. NESBIT, R. M., PAZZOS, R., and CUMMINGS, R. H. [Univ. of Michigan, Ann Arbor, Mich.] *J. Urol.*, **52**:570-574. 1944.

The authors compare a series of 75 prostatic cancer patients treated by castration, with one of 50 treated by diethylstilbestrol, and conclude: There was no significant difference in subjective response; objective changes suggesting regression were significantly greater in the castration group; neither form of therapy was prophylactic against the appearance of, or increase in, metastases. One patient with metastases and an elevated serum acid phosphatase level died of the disease even though the serum acid phosphatase was reduced to normal limits by stilbestrol; the osseous metastases, however, at the time of death contained considerable acid phosphatase. Hence, the authors believe the serum acid phosphatase level is not a reliable index of metastatic activity. One person who failed to benefit from castration improved after stilbestrol therapy.—V. F. M.

Metastasis in the Epididymis from Cancer of the Prostate: Case Report. HUMPHREY, M. A. [Ellis Hosp., Schenectady, N. Y.] *J. Urol.*, **51**:641-642. 1944.

An instance of this rare condition is described.—V. F. M.

Secondary Carcinoma of the Testicle Following Carcinoma of the Prostate. HELFERT, I., and PINCK, B. M. [New York Post-Grad. Med. Sch. and Hosp., New York, N. Y.] *J. Urol.*, **51**:635-640. 1944.

Metastatic tumors to the testis are rare. A case of testicular metastasis from prostatic carcinoma is described. —V. F. M.

Tumors of the Testis. Analysis of Fifty Cases. HELLWIG, C. A. [St. Francis Hosp., and Sedgwick Co. Tumor Clin., Wichita, Kans.] *Urol. & Cutan. Rev.*, **48**:538-544. 1944.

Without serial sections the author found evidences of teratoma in 24 of 50 instances of testis tumors, but the original diagnoses were solid carcinoma, adenocarcinoma, or choriocarcinoma. Hence, he favors Ewing's opinion

that all testis neoplasms are actually teratomatous. A definitely positive result in the Aschheim-Zondek test has grave prognostic significance. In 1 patient, however, with a positive A-Z reaction, the reaction became negative after surgery and irradiation, despite widespread metastases. The histology can not be correlated with the results of the A-Z test. The author warns against too great enthusiasm over the use of x-radiation, since Cabot and Benkson reported 58.8% and 41.7% of 5 year cures for seminoma and adenocarcinoma respectively in a series of 37 cases treated by orchidectomy alone. The 5 year survival rate of the author's series, in which treatment was by orchidectomy, irradiation, or both, was 38.9%.—V. F. M.

Embryonal Adenocarcinoma of the Testicle in an Infant—Case Report. MATASSARIN, F. W. [Station Hosp., Fort Benning, Ga.] *J. Urol.*, **52**:575-577. 1944.

This is a case history of a 7 month old infant.—V. F. M.

Prognosis in Teratoma Testis. BARRINGER, B. S. [Memorial Hosp., New York, N. Y.] *J. Urol.*, **52**:578-585. 1944.

The author reviews 69 cases and concludes that adenocarcinoma with metastases has a better prognosis than metastasizing seminoma, in spite of the fact that seminoma is the more radiosensitive. The liver was involved in 75% of patients having lung metastases.—V. F. M.

A Critical Review of the Pathogenesis of Chorioma Testis and a New Theory. PETILLO, D. [New York, N. Y.] *Urol. & Cutan. Rev.*, **48**:53-66. 1944.

The hypothesis is advanced that chorioma testis and chorioma ovarii are not teratomas but "embryo-choriomas" that arise from the sex reversal of germ cells. Thus, in the testis, some of the male germ cells become converted into oocytes, which collide with the normally present spermatozoa (autofertilization) to form embryos that ultimately die and degenerate, leaving the chorion. In the ovary, correspondingly, some of the ova become converted to spermatozoa, and similar autofertilization takes place. —M. H. P.

Hemangioma of the Testis. KLEIMAN, A. H. [Vet. Admin. Facility, Hines, Ill.] *J. Urol.*, **51**:548-550. 1944.

Testicular hemangioma occurring in a 51 year old patient is described.—V. F. M.

Rhabdomyosarcoma of the Spermatic Cord. SHIVERS, C. H. DET. [Atlantic City Hosp., Atlantic City, N. J.] *J. Urol.*, **52**:266-274. 1944.

A case report, illustrated.—V. F. M.

Unusual Tumors and Secondary Carcinomas of the Penis. Review of the Literature and Report of a Case. WATTENBERG, C. A. [Washington Univ. Sch. of Med., and Barnes Hosp., St. Louis, Mo.] *J. Urol.*, **52**:169-175. 1944.

This is a tabulation and review of unusual penile tumors, and a report of a case of carcinoma arising in the urinary bladder and metastasizing to the corpora cavernosa of the penis.—V. F. M.

Sarcoma of the Penis. LEVANT, B. [Pittsburgh, Pa.] *J. Urol.*, **52**:63-66. 1944.

A primary sarcoma, "probably of the reticulum cell type," occurring in the corpus cavernosum in a 29 year old Negro is described.—V. F. M.

Primary Fibrosarcoma of the Penis: Review of the Literature and Report of a Case. WATTENBERG, C. A. [Washington Univ. Sch. of Med., Barnes Hosp., and Washington Univ. Clin., St. Louis, Mo.] *J. Urol.*, **51**:543-547. 1944.

The literature contains reports of only 7 primary fibrosarcomas of the penis. An additional case is described. The prognosis is usually good.—V. F. M.

Penile Horn. TAYLOR, J. A. [New York, N. Y.] *J. Urol.*, **52**:611-614. 1944.

Horn formation by a penile cancer is described.—V. F. M.

URINARY SYSTEM—MALE AND FEMALE

Renal Malignancy and the Prostatic Patient. KEEN, M. R. [Huntington, N. Y.] *J. Urol.*, **52**:109-114. 1944.

This is a discussion, with illustrative cases, of coincidental renal neoplasm and prostatism.—V. F. M.

Tumors of the Kidney. WHITMORE, E. R., LECOMTE, R. M., and BENNETT, W. W. [Georgetown Univ. Med. Sch., Washington, D. C.] *Urol. & Cutan. Rev.*, **48**:157-164. 1944.

A classification of renal tumors is presented with illustrative cases.—V. F. M.

Primary Adenocarcinoma of the Kidney—A Clinical and Pathological Study. CASILLI, A. R. [Elizabeth Gen. Hosp., Elizabeth, N. J.] *Urol. & Cutan. Rev.*, **48**:549-552. 1944.

An analysis of 21 instances of renal adenocarcinoma.—V. F. M.

A Twelve-Year Cure Following Nephrectomy for Adenocarcinoma and Lobectomy for Solitary Metastasis. BARNEY, J. J. D. [Boston, Mass.] *J. Urol.*, **52**:406-407. 1944.

A female with adenocarcinoma of the kidney and a lung metastasis was treated by nephrectomy and lobectomy. Twelve years later she is well and without evidence of disease.—V. F. M.

Wilms' Tumor: A Case Report. MCGEE, H. J. [Buffalo, N. Y.] *J. Urol.*, **52**:489-491. 1944.

The author advocates surgical treatment, and describes a case in which failure to operate promptly may have been responsible for the fatal outcome.—V. F. M.

Adenomyosarcoma of the Kidney in the Adult (Wilms' Tumor). WOOD, D. A. [Nav. Hosp., Mare Island, Calif.] *J. Urol.*, **51**:235-244. 1944.

The case of a 45 year old Filipino with extensive metastases from a Wilms' tumor is presented and illustrated. Twenty-three previous instances of Wilms' tumor in adults are recorded in the literature.—V. F. M.

ORAL CAVITY AND UPPER RESPIRATORY TRACT

Carcinoma of the Lip. ECKERT, C. T., and PETRY, J. L. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America*, **24**:1064-1076. 1944.

No local recurrence from carcinoma of the lower lip after 5 years occurred in 94% of 299 patients treated by V-excision, in 90% of 121 patients given surface radium therapy, or in 36.3% of 11 patients with extensive

lesions treated by radium needles. Among patients with carcinoma of the upper lip, the rate of 5 year survival without recurrence at the primary site was 50% after either surgery (12 cases) or radium therapy (6 cases). Biopsy is the only reliable method of diagnosis. A routine suprahyoid neck dissection should be performed in every early case of cancer of the lip, even if no lymph nodes are palpable, and meticulous follow-up examinations should continue throughout the life of the patient.—J. L. M.

The Surgical Management of Cancer of the Larynx. Sherwin, C. F. [Barnard Free Skin and Cancer Hosp., and St. Louis Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America,* **24**:1089-1099. 1944.

The primary operative mortality from laryngectomy at the Barnard Free Skin and Cancer Hospital, which was 55.5% in 9 cases during 1920 to 1929, was reduced by improvements in technic to 23.5% in 21 cases during 1930 to 1944. In the author's private practice and in his practice in other hospitals, the primary operative mortality from laryngectomy has been 15.8% in 16 cases; of the 13 survivors, 8 were still alive and well at the time of writing, up to 17 years after operation. Usually 50% of the patients surviving the operation can be expected to live 5 years or more. The surgical technic and rehabilitation are discussed.—J. L. M.

Gastrointestinal Tract

Further Observations on Carcinoma of the Stomach in a Large General Hospital with Special Reference to One Hundred Thirty-Four Non-Surgical Fatalities from Charity Hospital of Louisiana at New Orleans. Boyce, F. F. [New Orleans, La.] *New Orleans M. & S. J.,* **97**:217-227. 1944.

A reminder that few permanent cures are obtained in the average hospital. There has been slight improvement in the mortality statistics in the past few years, but accuracy of diagnosis is still far from satisfactory, and the disease is not being recognized in its incipient stage. Differentiation from ulcer may be difficult, and negative roentgenologic findings should not be regarded as final in the face of a typical clinical picture. The author believes gastric ulcer in middle life should be explored for accurate diagnosis.—E. E. S.

Diagnostic Delay in Gastric Carcinoma. Engel, G. C. [Lankenau Hosp., Philadelphia, Pa.] *Pennsylvania M. J.,* **48**:126-129. 1944.

A discussion of deaths from carcinoma of various organs, and early symptoms, operability, and causes for high mortality in gastric carcinoma. Cancer of the stomach should be suspected in any patient with a history of tired and weak feeling, loss of appetite (especially for meat), or indigestion of 4 weeks' duration or more.—J. L. M.

Aberrant Pancreatic Tumor in the Duodenal Wall. Brown, S., Flachs, K., and Wasserman, P. [Jewish Hosp., Cincinnati, Ohio] *Radiology,* **43**:385-386. 1944.

A case report, presented because of the rarity of the lesion and the interesting x-ray findings.—R. E.S.

Acutely Obstructing Carcinoma of the Colon. Gruenfeld, G. E. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *S. Clin. North America,* **24**:1126-1142. 1944.

A review of incidence, mechanical factors, diagnosis, preoperative therapy, and surgical technics.—J. L. M.

Adenoma of the Rectum. Womack, N. [Barnard Free Skin and Cancer Hosp., Washington Univ. Sch. of Med., and Barnes Hosp., St. Louis, Mo.] *S. Clin. North America,* **24**:1143-1150. 1944.

Three cases are presented to show that microscopic examination of a small fragment of an adenoma of the rectum is entirely inadequate for diagnosis. Since this tumor so frequently becomes malignant, it should be completely excised, with the underlying rectal wall. Adequate microscopic study can then be made, and if malignant invasion is found, further surgery may be performed.—J. L. M.

Squamous Cell Carcinoma of the Anus and Rectum. Keyes, E. L. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *S. Clin. North America,* **24**:1151-1161. 1944.

In a treatment period ending 5 years ago, 17 patients received surgery or radiation for squamous cell carcinoma of the rectum or anus, and 7 of them have survived cancer-free for 5 years or more. The characteristics, diagnosis, and treatment of the disease are discussed, with special reference to the 17 patients mentioned above and to 33 others who were untreated or who received therapy too recently for assessment of the 5 year cure rate.—J. L. M.

Peritoneum and Retroperitoneum

Zur Kasuistik der Mesenterialcysten. Ein Fall von Chylangiom. [On Mesenteric Cysts. A Case of Chylangioma.] Brattström, S. [Hälsingborg Hosp., Hälsingborg, Sweden] *Acta chir. Scandinav.,* **86**:308-314. 1942.

Three large chylous cysts in the mesentery, causing strangulation of the lower coil of the ileum in a boy from time to time during his seventh to ninth years of age, were excised, and proximate parts of the intestine were removed. Radical surgery is recommended for mesenteric cysts, since they may lead to serious complications. Photograph; photomicrograph.—M. H. P.

Large Cyst of the Omentum. Its Occurrence in a Three Year Old Child. Leach, W., and Holland, M. [Locust Mountain State Hosp., Shenandoah, Pa.] *Am. J. Dis. Child.,* **65**:920-921. 1943.

A case report.—C. J. M.

A Retroperitoneal Digit-Containing Teratoma. Gale, C. W., and Willis, R. A. [Kitchener Memorial Hosp., Geelong, Alfred Hosp., Prahran, and Univ. of Melbourne, Victoria, Australia] *J. Path. & Bact.,* **56**:403-409. 1944.

A teratomatous mass was excised from a thick-walled retroperitoneal cyst near the duodenum and pancreas in a 13 year old girl. It contained a well developed digit with two nails, a metacarpal bone, and fused phalanges. Twenty other reports of nail- and digit-bearing teratomas are referred to, and the unwarranted identification of "limbs" is criticized. Drawings; photographs; roentgenograms.—L. W. P.

Bone, Bone Marrow, Joints

Clinico-Pathological Conference. Haggart, G. E., Hare, H. F., and Marks, J. H. [Lahey Clin., and New England Deaconess Hosp., Boston, Mass.] *Radiology,* **43**:378-382. 1944.

A case of osteochondrosarcoma of the right ischium is reported.—R. E. S.

Capillary Hemangioma of Bone. Sherman, M. S. [Univ. of Chicago, Chicago, Ill.] *Arch. Path.,* **38**:158-161. 1944.

Case report.—J. G. K.

Chordomata: A Review of the Literature, with Report of a Sacrococcygeal Case. Faust, D. B., Gilmore, H. R., Jr., and Mudgett, C. S. [Walter Reed Gen. Hosp., Washington, D. C.] *Ann. Int. Med.,* **21**:678-698. 1944.

Comprehensive discussion of the literature, with report of a case in which a large chordoma extended from the sacrococcygeal region into the right lower quadrant of the abdomen and metastasized extensively to the lungs and adrenals. Roentgenograms; photomicrographs; photographs; 95 references.—J. G. K.

Multiple Diffuse Fibrosarcoma of Bone. Steiner, P. E. [Univ. of Chicago, Chicago, Ill.] *Am. J. Path.,* **20**:877-893. 1944.

An osteolytic fibrosarcoma of bone (No. 2032, Bone Sarcoma Registry) is described, in which the lesions appeared at approximately the same time in many bones, and in which the tumors, although highly infiltrative, retained the normal configuration of the bones. The distribution and extent of the sarcoma was that of the hemopoietic and reticuloendothelial areas in the skeleton. There were small metastases in many viscera. It is believed that this is an example of a medullary fibrosarcoma somewhat analogous to the myelomas, and that it, like them, might have had a multicentric origin.—Author's summary. (J. G. K.)

Multiple Myeloma in a Fifteen-Year-Old Boy. Rubinstein, M. A. [Montefiore Hosp., New York, N. Y.] *New York State J. Med.,* **44**:2491-2494. 1944.

A case of plasma-like cell multiple myeloma in a boy of 15 is reported. It illustrates the importance of bone marrow aspiration in the diagnosis of myeloma, and emphasizes the point that youth should not rule out the possibility of this disease.—J. L. M.

Primary Malignant Neoplasm of the Shoulder Joint with Report of a Case. Olin, H. A. [Woodlawn Clin. and Hosp., Chicago, Ill.] *Radiology,* **42**:359-367. 1944.

The anatomy and physiology of the shoulder joint are reviewed, and a case report of fibrosarcoma of this joint is presented with roentgenograms, diagrams, and photomicrographs.—R. E. S.

Blood Vessels

Tumors of Blood Vessels. Stout, A. P. [Columbia Univ. Coll. of Physicians and Surgeons, and Presbyterian Hosp., New York, N. Y.] *Texas State J. Med.,* **40**:362-365. 1944.

A general discussion.—J. L. M.

Leukemia, Lymphosarcoma, Hodgkin's Disease

Manifestations of Hemolytic Phenomena and Infectious Mononucleosis in a Case of Lymphatic Leukemia. Feldman, F., and Yarvis, J. J. [Kings Co. Hosp., Brooklyn, N. Y.] *New York State J. Med.,* **44**:1693-1694. 1944.

A case report.—J. L. M.

Chloroma and the Origin of its Green Color. Zeldenrust, J., Veer, W. L. C., and Nota, J. H. W. [Leiden Univ., Leiden, Netherlands] *Nederl. tijdschr. v. geneesk.,* **87**: 875-890. 1943. From abstr. in *Chem. Zentralbl.,* **II**:1287. 1943.

In a 3½ year old child with myeloid chloroleukemia, the green color of the abnormal growths was found to be due to protoporphyrin. This finding confirmed a previous report by Thomas.—M. H. P.

Malignant Lymphomas of the Spinal Epidural Space. Verda, D. J. [Barnard Free Skin and Cancer Hosp., and St. Louis City Hosp., St. Louis, Mo.] *S. Clin. North America,* **24**:1228-1244. 1944.

A general discussion, illustrated by 2 case histories, roentgenograms, drawings, and diagrams. The subjects covered comprise the anatomy of the spinal epidural space, the modes of involvement of this region by lymphomas, and the symptoms, diagnosis, forms, and treatment of these tumors. Laminectomy with removal of the spinal epidural lymphoma, followed by roentgenotherapy in cases in which the spinal epidural space is the primary region of involvement, offers moderately favorable prognosis. Roentgenotherapy alone is indicated only when compression of the cord is a terminal manifestation resulting from an infiltrating lymphomatous process from regional lymph nodes.—J. L. M.

Clinico-Pathological Conference. Shrader, E. L., Sante, L. R., and Anderson, W. A. D. [St. Louis Univ. Sch. of Med., St. Louis, Mo.] *Radiology,* **43**:293-296. 1944.

A case of lymphoblastoma belonging to the Hodgkin's sarcoma type is reported.—R. E. S.

Co-Existence of Chronic Lymphogranuloma and Cancer. Guzman, L. [Inst. Nacional del Radium, Santiago, Chile] *Radiology,* **41**:151-156. 1943.

The incidence of lymphogranuloma associated with cancer is low. Nine cases in a total of 12,546 patients with cancer and allied diseases are reported.—R. E. S.

Intrathoracic Hodgkin's Disease. Wolpaw, S. E., Higley, C. S., and Hauser, H. [Cleveland City Hosp., and West. Reserve Univ. Sch. of Med., Cleveland, Ohio] *Am. J. Roentgenol.,* **52**:374-387. 1944.

Of 55 proved cases of Hodgkin's disease in which chest roentgenograms were available, 35 showed intrathoracic involvement. The varied manifestations were correlated with the distribution of lymphoid tissue within the chest; mediastinal, parenchymal, pleural, osseous, and cardiac types were described. Seventeen of 23 patients with intrathoracic Hodgkin's disease showed favorable response to x-ray therapy.—E. H. Q.

Hodgkin's Disease of the Skin. Levin, O. L., and Behrman, H. T. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.,* **11**:207-210. 1944.

A wide variety of cutaneous manifestations have been placed under the heading of the lymphomatoid diseases

of the skin. The skin phenomena that occur in Hodgkin's disease may be put in one of two groups. Those in the first group are the more common and consist of nonspecific lesions that are neither histologically nor clinically pathognomonic of the disease. (Of their symptoms, itching is the most frequent. It may be extremely intense and unaccompanied by any changes whatsoever in the skin.) The second group includes lesions that show the specific and pathognomonic histologic characteristics of the disease in the skin. These lesions are comparatively rare; they are usually tumors or plaques. The cutaneous changes may antedate the general symptoms and signs or occur concurrently with them. In the case reported, palliation followed x-ray therapy, in both the systemic and the cutaneous manifestations. However, the cutaneous lesions became radioresistant and formed firm keloidal masses.—A. Cnl.

An Unusual Case of Cutaneous Hodgkin's Disease with Terminal Blood Stream Spread. Bersack, S. R. [Vet. Admin. Facility, Hines, Ill.] *J. A. M. A.*, **126**:1025-1026. 1944.

A case report. The cutaneous lesions presented the unusual feature of ulceration. The author suggests that the occurrence of terminal blood stream spread without evidence of embolic phenomena is consistent with a virus etiology of Hodgkin's disease.—M. E. H.

Pancreas

Pancreatic Carcinoma Involving Urinary Tract (Report of Two Autopsy Cases). Putschar, W. G. J., and Irwin, G. G. [Charleston Gen. Hosp., Charleston, W. Va.] *Urol. & Cutan. Rev.*, **48**:544-548. 1944.

Two clinical case reports, with autopsy findings, demonstrate invasion of the right ureteropelvic region by pancreatic carcinoma. The literature on the subject is reviewed.—V. F. M.

Thyroid

Histologically Benign Thyroid Tumors Producing Paraplegia and Chyluria. Tucker, H. A. [Gorgas Hosp., Canal Zone] *West. J. Surg.*, **52**:467-469. 1944.

Two case reports of so-called benign metastasizing colloid goiter.—M. E. H.

CANCER CONTROL AND PUBLIC HEALTH

Origin and Avoidance of Cancer. Lazarus, P. *Bull. Soc. fribourgeoise d. Sc. naturelles*, **35**:74-86. 1941. From abstr. in *Chem. Zentralbl.*, **II**:723. 1943.

After a review of the modes of origin of cancer, a 10 point program for the prevention and control of this disease is outlined. The recommendations concern: responsible individual hygiene; avoidance, from childhood on, of prolonged or repeated exposure to irritants in the environment; industrial hygiene; social hygiene; education by private physicians and public health authorities; special curative prophylaxis of precancerous lesions; early radical elimination of all *puncta minoris resistentiae;* immediate eradication of early cancer; thorough care of persons subjected to surgery or irradiation; and periodic physical examinations.—M. H. P.

Industrial Health. Merewether, E. R. A. *Annual Report of the Chief Inspector of Factories for the Year 1943*, 38-56. 1944.

A filling-machine operator aged 57, for 43 years in a factory manufacturing sheep dip containing sodium arsenite, died of carcinoma of the lung. "Three similar cases of pulmonary carcinoma occurring in arsenical sheep dip workers have been notified since 1939."

In Great Britain during the year, 160 (15 fatal) cases of epithelioma of the skin were notified, 111 due to pitch or tar, 48 to mineral oil, and 1 to shale oil. "Of the 111 cases due to pitch or tar, 20 occurred in patent fuel makers, 61 in tar distillers, 10 in coke oven workers, 13 in gas workers, 2 in stevedores loading and unloading pitch at wharfs, 2 in makers of electric brushes, and 1 each in proofing of brattice cloth, making of coalite (coal carbonisation), and handling creosote." A large number of these cases, when notified in the early stages, receive radiological treatment.—E. L. K.

Treatment of Cancer. Directions for the Use of Record Cards. Parts I and II. Ministry of Health, National Radium Commission [London, England]. See also *Lancet*, **247**:572-573. 1944.

The Ministry has issued as a booklet a series of suggestions drawn up by the Radium Commission for the guidance of those responsible for keeping records of patients suffering from cancer. A form-card is arranged to give details of history, diagnosis, clinical findings, histology, treatment, and follow-up.—E. L. K.

Recording of Cancer Cases. Smithers, D. W. [London, England] Letters to the Editor. *Lancet*, **247**:643. 1944.

The author points out that the mass of data collected on the cards described in the preceding abstract will be difficult to handle unless a punch-card system with mechanical sorting is adopted. The card is criticized also in that it provides for the calculation of survival rates but not for a "disease-free rate" associated with any one method of treatment.—E. L. K.

Administration of the National Cancer Institute Act, August 5, 1937, to June 30, 1943. Marshino, O. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:429-443. 1944.

This is a summary of the activities of the National Cancer Institute dealing mainly with: (1) the functions of the National Advisory Cancer Council, (2) radium loans by the National Cancer Institute to other institutions, (3) the establishment of traineeships in the diagnosis and treatment of cancer in various medical centers, (4) the establishment of research fellowships in the National Cancer Institute, (5) the *Journal of the National Cancer Institute*, (6) a summary of the nature of the fundamental cancer research being carried on at the National Cancer Institute, (7) the work of the Clinical Research Center at the U. S. Marine Hospital in Baltimore, Md., (8) a summary of the statistical studies on cancer being done at the National Cancer Institute, (9) notes on the examination of cancer cures, and (10) the function of the education and information services.—R. B.

Book Reviews

VENTURES IN SCIENCE OF A COUNTRY SURGEON. Arthur E. Hertzler. Halstead, Kansas. 1944. xi+304 pages; 19 illustrations.

In this, his latest book, the Horse and Buggy Doctor takes us from Virchow's laboratory, in Berlin, where he began his studies on the peritoneum, to Halstead, Kansas, where he still practices his art; a matter for heartfelt congratulation to the good citizens of that community.

He takes us, also, from wound healing through inflammation, anatomy, anesthesia, surgery, and the treatment of sciatica, all the way to the writing and illustrating of books and the assembling of a library. His experimental material ranged from rabbits' ears to his own leg.

Students of cancer will be interested to learn that in his investigations on the etiology of tumors he anticipated Fischer-Wasels in the subcutaneous injection of fat stains, and will welcome him to their circle with open arms and smiles of sad reminiscence when they read about his unsuccessful theory of malignancy.

As there cannot have been much time in such a busy life for the study of philosophy it may not be too ungracious in the reviewer to point out that it was not Kant, but Descartes, who said: "I think, therefore I am."

Wm. H. Woglom

MITOSIS. THE MOVEMENT OF CHROMOSOMES IN CELL DIVISION. By Franz Schrader. New York: Columbia University Press. 1944, 8vo, 110 pages. Price $2.00.

Professor Schrader is to be complimented for his courage in plunging into the chaotic array of facts and fancies that abounds in the literature on mitosis. He has done a heroic job in assembling so many of the multitudinous accounts on the subject, both factual and hypothetical. The author is well qualified to do so, from the considerable amount of his own observational research and the great deal of thinking he has devoted to the subject.

Keenly conscious of the obscurity that exists concerning the basic significance of the process of karyokinesis, he points out in his introduction that his survey has been made primarily for the purpose of outlining the present status of the problem.

As a working method the author takes the attitude that the subject of cell division need not be regarded as a whole, and that a final solution may be furnished by an analysis of any one of the mitotic processes to the exclusion of others. Hence the many features, which are treated largely from a formalized, morphological point of view, are considered separately.

He cites, as an illustration of the independence of at least some of the processes, findings from which it has been asserted that the cleavage of the cytoplasm of a cell is possible in "the complete absence of nuclear material." However, it is necessary to point out that the results described have been obtained only from experiments on eggs that have undergone maturation, the asserted enucleation being the removal of the relatively small female pronucleus. Yatsu and E. B. Wilson long ago demonstrated the profound difference in behavior of the cytoplasm of the egg before and after breakdown of the germinal vesicle. This feature has been more recently confirmed and extended by the writer of this review. During maturation the nuclear material, arising from the germinal vesicle, or nucleus of the immature egg, impregnates the cytoplasm, which therefore cannot be regarded as somatic cytoplasm and certainly is not free of nuclear material. I emphasize this point because, thus far, there is every reason to expect that an understanding of the various intracellular processes will require that they be considered only as interdependent reactions of an integrated whole.

In describing cellular events it is well recognized that it is far better to deal with them while they are occurring in the living cell. Protoplasm is a native protein system that is profoundly affected and is bound to produce artefacts when the protein complexes are denatured by fixing agents. A case in point, which Schrader realizes in discussing astral rays, but less so in discussing spindle "fibers," is the following: Flow, in a living system, may be visible as such and may show birefringency because of the directed arrangement of the suspended colloidal constituents in the flow. But this is a far cry from the fibers demonstrated in fixed material.

The book is intended primarily for the cytological specialist. Its author makes frequent comments, but generally keeps himself as an objective spectator throughout, interested in marshaling the published data for those who may use them in their own experimentation. The general reader will have to refer to classic texts for definitions of many technical terms, but with this provision he should find much of interest. The investigator of cancer who is interested in the effects of agencies on mitotic events will find little. The main stress is on data and on proposed assumptions for explaining the movements of the chromosomes. The latter is so much the essence of the book that the subtitle might better have served as the title.

A rehearsal of some of the sections will indicate the general nature of the contents. Under "Structure" there is a discussion, occupying 38 pages, of what is to be seen in living and in fixed cells, with arguments for and against the "reality" of spindle fibers, together with a discussion on the nature and origin of the spindle apparatus. The section on "Hypotheses of Mitosis" occupies pages 39 to 74 and deals with movements and splitting of chromosomes, to be explained variously as due to contraction and expansion, viscosity and hydration, electrostatics, diffusion, and streaming hydrodynamics. The arrangement of tactoids is discussed, and the last 4 pages deal with chromosome autonomy. Pages 75 to 83 are devoted to "Related Problems" dealing with special cases such as the pairing of chromosomes, attraction, the bouquet stage, and a brief note on the condition of chromosomes in the "resting stage." The section, "Conclusion," occupies less than 3 pages, and is all too short for epitomizing the amount of detail covered by the book. Schrader rightly criticizes most of the proposed explanations of mitotic events on the ground that they tend to be limited too much to one or another assumed force, and he presents the idea that in all probability mitosis is a combination of a great complexity of different mechanisms. How-

ever, the question arises whether he does not go to the other extreme by attempting to analyze the several aspects of mitosis to the exclusion of the whole.

He has realized that our knowledge of mitosis and mitotic behavior is not sufficiently crystallized to permit a critical evaluation of the subject treated. The book, therefore, is not a monograph, but should be considered rather as an extensive review article. A good beginning has been made of presenting an excellent morphological setting for further investigation in which a more physicochemical aspect, based on experimentation with the living object, should be stressed.

Over 400 references are cited in the literature, the great majority being from 1925 to 1943 inclusive. The book is well indexed.

ROBERT CHAMBERS

THE BIOLOGICAL BASIS OF INDIVIDUALITY. Leo Loeb. Springfield, Ill.: Charles C. Thomas. 1945. xiii + 711 pages. Price $10.50.

The author distinguishes two types of individuality.

Mosaic individuality represents the sum of the particular organ and tissue characteristics (*organ and tissue differentials*) that distinguish kidney from thyroid, say, and determine structure, metabolism, and motor and psychic activities. These multiple characteristics are combined into a composite, or mosaic, which is the indivdual.

The second type, which may be called the essential individuality, is characterized by the presence of a chemical factor, the *individuality differential,* which is not restricted to certain parts of the organism as are the organ and tissue differentials, but is common to all and different from its counterpart in every other individual. This concept emphasizes the oneness of the individual, which depends upon the presence of a common and unique factor in all his essential parts.

Just as individuality differentials characterize individuals so do species, order, and class differentials, each with a specific chemical constitution, distinguish the larger groups of organisms. All these, together with the individuality differentials, may be combined under the name *organismal differentials,* and contrasted with the organ and tissue differentials that compose the mosaic individuality. Of them all, the individuality differential is the highest and finest.

In general the organismal differentials can be analyzed by transplantation and by serological methods, each of which has its own sphere where it can be applied to greatest advantage.

Individuality differentials, with which this book is particularly concerned, can be demonstrated by transplantation between more or less closely related animals, a procedure that the author has made peculiarly his own during the course of a long and uncommonly fruitful life. His experiments have shown that the cells of the host recognize the most subtle distinctions between individuality differentials, and that of all the body cells it is the lymphocyte that is able to sense the finest degrees of similarity or dissimilarity between host and graft.

With these observations as a basis Dr. Loeb enters upon a discussion of immunity, regeneration, tissue equilibrium, fertilization, tumors, blood groups, Forssman antigens, idiosyncrasy, anaphylaxis, serological methods, toxins, and evolution. The volume closes with a philosophical consideration of man and his environment, whose final conclusion is that environment has become a preponderating influence, and largely determines his fate.

To readers of this journal the section on tumors will naturally be the most interesting. As everyone must know, Dr. Loeb has consistently regarded the tumor problem from the standpoint of tissue growth, and has continually emphasized the parallelism between neoplasms and normal tissues after transplantation. In either case, the less closely related are donor and recipient the more intense will be a hostile reaction against a graft. As a result of his own work and that of many others, the analogies between tumors and normal tissues continued to accumulate until it became clear to all that the immunity induced by transplanted new growths is not directed against them specifically, because they are tumors, but nonspecifically and merely because they are composed of cells more or less alien to the new host.

Since the organismal differentials in tumors have proved to be essentially the same as those in normal tissues, the features characteristic of neoplasia must be referable to something else. But those who continue to cherish the hope that tumors will be found to contain other antigens besides their organismal differentials will realize that Dr. Loeb has little encouragement to offer. Conceding that there may be such substances, he nevertheless regards present evidence for the existence of a specific cancer antigen as vague and conflicting.

In common with their organismal differentials, the individuality differentials of tumors are essentially similar to those of the normal tissues.

In the discussion on etiology the somatic mutation hypothesis is dismissed as faulty and probably wrong, the virus hypothesis as applicable only to some neoplasms. The author's preference is for the hypothesis that gradual increases in growth momentum lead to intermediate stages of sensitization and ultimately to irreversible, malignant proliferation; perhaps through the mediation of an autocatalytic growth substance.

The malignant transformation depends upon conditions that are comparable to, though not necessarily identical with, changes in organ and tissue differentials. Tumor cells, even more than regenerating ones, have properties different from those of normal cells, certainly, but the alterations that take place during the malignant transformation are in all probability not specific, in the sense that they depend upon changes in the organismal differentials of the affected tissues that would result in the development of antibodies against the individuality differentials of the tumor cells. The transformation is a consequence of interaction between genetic factors, transmitted by the germ cells, and certain stimulating influences.

This book, like others that the reviewer has seen from its publisher, is unusually free of misprints. Though the author modestly calls his bibliography "very incomplete," it contains nevertheless some 900 references, and at least in the tumor section very few pertinent articles, if any, can have been overlooked. The index is adequate to its purpose. There are no illustrations.

WM. H. WOGLOM